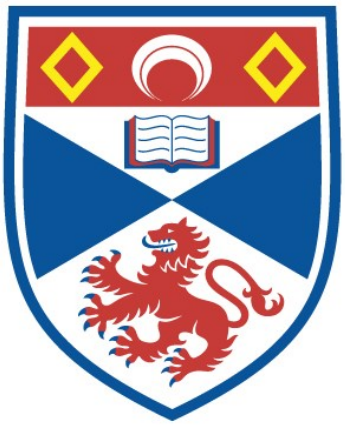
QUANTITATIVE EXAMINATION OF SEED OILS BY CHROMATOGRAPHIC PROCEDURES

Rajashekhar Chanabasappa Badami

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1962

QUANTITATIVE EXAMINATION OF SEED OILS

BY

CHROMATOGRAPHIC PROCEDURES.

being a Thesis

presented by

RAJASHEKHAR CHANABASAPPA BADAMI, B.Sc., M.Sc.

to the

UNIVERSITY OF ST. ANDREWS

in application for

THE DEGREE OF DOCTOR OF PHILOSOPHY.

May 1962.

Th 5090

DECLARATION.

I hereby declare that the following thesis is based on results of experiments carried out by me, that the Thesis is my own composition and that it has not previously been presented for a Higher Degree.

The research was carried out in the Chemical Research Laboratories of the United College in the University of St. Andrews, under the direction of Dr. F. D. Gunstone.

CERTIFICATE.

I hereby certify that Mr. Rajashekhar Chanabasappa Badami has spent nine terms at research work under my supervision, has fulfilled the conditions of Ordinance 16 (St. Andrews), and that he is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Research Supervisor.

UNIVERSITY CAREER.

I entered the Karnatak College, Dharwar, University of Bombay, in 1938, pursued a recognised course for graduation in Science and graduated B. Sc. in 1942, M. Sc. (by research on seed oils) in 1947.

I was admitted as a Research student in the United College of St. Salvator and St. Leonard, University of St. Andrews, in January 1960.

I was appointed Demonstrator in Chemistry in 1945, Lecturer in Chemistry in 1947, in the Karnatak College, Dharwar, University of Bombay. In 1956, the Government of Mysore appointed me to a Gazetted post in the Karnatak College, Dharwar, Karnatak University.

I published 3 papers in the Journal of the Indian Chemical Society in 1949 and 5 short notes in the Proceedings of the Indian Science Congress Association (1952-1955).

In 1949, I was elected to the first Senate of the Karnatak University, for a period of 5 years.

I am a Fellow of the Indian Chemical Society and a member of the American Oil Chemists' Society.

PUBLICATIONS BASED ON THE WORK REPORTED IN THIS THESIS.

1. Vegetable Oils. (X). Examination of Component Acids of *Argemone mexicana* Seed Oil by Reversed-phase Partition Chromatography.
   By R.C.Badami and F.D.Gunstone, J.Sci.Fd.Agric., 1962, 13, 255.
2. The Component Acids of *Holoptelea integrifolia* Seed Oil.
   By R.C.Badami, J.Sci.Fd.Agric., 1962, 13,
3. Vegetable Oils. (XI). The Component Acids of *Boleko (Isano)* Oil
   (To be published).
4. Vegetable Oils. (XII). The Component Acids of *Vernonia* Seed Oils.
   (To be published),
5. Vegetable Oils. (XIII). The Component Acids of *Amburana* Seed Oil.
   (To be published).

ACKNOWLEDGMENTS.

I wish to thank Dr. F.D.Gunstone for his keen interest in this work and for his suggestions, criticism and discussions during the course of this work. I am also grateful for his constantly available advice.

I also wish to thank Professor J.Read F.R.S., for the provision of research facilities.

I am grateful to Dr. M.L.Meara for supplying Amburana (mamona) seeds and to Sir G.Taylor (Royal Botanic Gardens) for identifying them; to Mr. G.T.Bray (Tropical Products Institute) for supplying seeds (V.nigritana, V.amygdalina, V.colorata, V.biafrae and V.camporum; and also Argemone mexicana (Jamaican)); to Sri I.M.Qureshi (Silviculturist to the Govt. of Bombay) for supplying V.cinerea seeds; to Dr. S.Krishna (Scientific Liason Officer, India House) for supplying Argemone mexicana (Indian) seeds; to Dr. S.H.Bell (Paint Research Station) for supplying boleko (isano) oil; and to Sri J.D. Mascarhnas (Divisional Forest Officer, Dharwar) for supplying Holoptelea integrifolia seeds. I am grateful to Dr. P.J.Sykes for his help in the early stages of this work; to Mr. Z.M.Zochowski for the infrared spectra of Vernonia oils; to Mr. R.Morris for the photographs and for help from time to time. I am very grateful to my research

colleagues, specially to Dr. G.D.Baker, who shared the research room with me, for his cooperation and warm hearted friendship and to Mr. B.B.Molloy, for his valuable and ungrudging help in preparing the diagrams. I am also grateful to Mr. T.Norris and other members of the Chemistry Department for help from time to time, and wish to thank them all.

Finally, I wish to thank the Government of Mysore and the Karnatak University for the grant of Study Leave (without allowances) which enabled me to devote my time to this research.

CONTENTS.

(b)

****

The application of chromatography to the quantitative analysis of mixed fatty acids.

Introduction

In spite of the tremendous progress, in recent years, in the methods of analysis of fatty acids and other lipids, the total analysis of fats still remains a major problem, because of the great variety and complexity of fatty acids found in nature. The quantitative isolation and estimation of the component acids of a fat is a difficult and tedious process. Recent analytical studies[1,2] have shown that most fats contain a great variety of fatty acids some of which occur in low concentrations. Since the lower limit of detection of individual components has been greatly diminished and the separation of individual components improved, even trace components can now be detected and measured in a fatty acid mixture. The investigator now has at his disposal a choice of rapid and precise methods. But there is no single method for the accurate analysis of all fatty acids in a complex fatty mixture; sometimes the method is not selective enough, sometimes the concentration of the material in the sample is too low. A combination of methods is usually employed.

The classical methods of analysing fatty acid mixtures

were based mainly on crystallisation of acids or their salts and on distillation. These have been reviewed by Gunstone.[3] By the careful use of these methods a large amount of information has been obtained but they have now been largely (though not entirely) replaced by other procedures requiring less material, less time and less skill but usually giving more information.

In the last few years, there have been great changes in analytical chemistry mainly through the development of instrumental techniques and of improved methods of separation. The most important advances in the analysis of fatty acids have been based on chromatographic techniques which will now be reviewed.

Chromatography may be defined as a separation technique by which components in a mixture are distributed between two phases; one phase is stationary and has a large surface; the other phase is mobile. The substances to be separated move at different rates depending on their relative affinities for the stationary and mobile phases.[4]

Chromatographic techniques can be subdivided according to whether the separation process depends essentially on adsorption or partition (in most experimental separations both are involved but one usually predominates) and on whether the technique is applied in a column or in a thin layer, thus:

	Column	Thin layer
Adsorption	Adsorption chromatography	Thin layer chromatography
Partition	Partition chromatography	Paper chromatography
	Gas-liquid chromatography	

Adsorption chromatography

In this method, the fatty materials to be separated are adsorbed on columns of alumina, silica or other inorganic materials, and then desorbed (eluted) with appropriate solvents of increasing polarity.

The fatty mixture, in an appropriate solvent, is allowed to filter through the column and the constituents of the mixture are adsorbed on to the column in different zones and the adsorbed zones are separated one from the other by elution from the column, one at a time. The least adsorbed substance lies in the lower zone and is eluted first, and the most adsorbed substance lies in the upper zone and is the last to be eluted.

The relative positions of the zones can be located 5-10 whilst still on the column and the zones can be eluted one at a time, thus dividing the eluates into fractions, each containing (ideally) one substance (constituent). The material from each fraction is recovered and the composition determined.

Adsorption chromatography has been applied to the separation, identification and estimation of individual fatty acids with varying degrees of success.[11-16] Some of the studies required large volumes of eluents and long period of time. For example, separation of myristic and stearic acids in a magnesia-petroleum ether system required 14 days.[15] Model mixtures which have been successfully separated include palmitic, stearic and oleic acids on alumina[17,18] and on activated charcoal,[8] and cis and trans isomers of unsaturated fatty acids have been analysed using a silica gel-petroleum ether system.[20] Dutton and Reinbold[19] investigated the separation of all the possible pairs from ethyl stearate, oleate, linoleate and linolenate. Pure methyl linoleate has been separated from cotton seed oil esters[21] and pure linoleate and linolenate esters from methyl esters of linseed and perilla oil.[22] Pure methyl arachidonate was isolated from adrenal lipids and the presence of eicosapentaenoic and docosapentaenoic acids also demonstrated.[23] Positional isomers of linoleic acid have been separated from partially hydrogenated linseed oil.[24] Oxidation products of methyl oleate[25] and linoleate,[27] and hydroxystearic acids from hydrogenation of autoxidised methyl linoleate[26] have all been isolated with good weight recoveries. Saturated and unsaturated fatty acids have

been measured based on the adsorptive separation of the products of oxidation of unsaturated acids from the saturated acids.[28] Cis 12:13 epoxyoleate (79-80 %) was isolated from Vernonia anthelmintica methyl esters on a silicic acid column.[29]

The proper separation by adsorption chromatography depends on selection of suitable adsorbents and solvent(s), careful location of zones, and separation of eluents into different fractions containing single substances. The method requires large volumes of eluents and long period of time.

The method is more concerned with separation and isolation rather than analysis and is not much used for the analysis of component acids.

Thin layer chromatography (T.L.C.)

The separation of fatty acid esters by adsorption on silica gel or other adsorbents in the form of a thin layer on a glass plate is described as thin layer chromatography.

The chromatoplates are prepared[30] by spreading thin layers(250 to 275u) of silicic acid on glass(20 x 20cm) using commercial fine grain(250 mesh) silica gel, containing plaster of paris(1 %). For reversed-phase partition T.L.C., tho thin layers of silica gel are further impreg-

nated with silicone,[31] undecane,[32] higher paraffins[33,34] or with squalene.[30]

Samples are applied to the plates in amounts of 0.5-1.0 mg., as approximately 1 %. solutions along one side of the plate 2 cm. from the edge, and the constituents separated by ascending elution with a suitable solvent system.[30] The positions of the components are determined by development with iodine to show up unsaturated components or with alphacyclo-dextrin and iodine, to show up saturated components. The spots can also be detected by spraying the plates with 50 %. sulphuric acid, and heating until all organic constituents are charred and appear as black spots; the rate of appearance and the initial colour of individual spots give qualitative information regarding the degree and type of unsaturation of the components. In the examination of long-chain fatty esters, one class of esters at a time can be made to migrate by varying the polarity of the eluting solvent.[35] The spots are identified by running standard esters side by side with the sample and comparing the Rf values.

Various methods have been suggested for the quantitative evaluation of T.L.C. Very few of these methods are exact and the better ones are not simple to operate.

Mixtures of methyl esters of fatty acids can be rapidly

and quantitatively separated in the form of their acetoxymercury methoxy derivatives, into saturated esters, monoenoates, dienoates and trienoates.[50] The original esters can be recovered after separation of their addition compounds. Vioque and Holman[36] have described the separation of fatty esters by T.L.C. and quantitative determination via their hydroxamic acids. Esters of different types are separated on silica gel plates, the spots are scraped from the plates, the esters are extracted from the silica gel and the iron hydroxamic acid complexes are formed. The amount of each ester class is then determined colorimetrically. Vioque and Holman suggest that this procedure may also be used to analyse mixtures of mono, di and triglycerides.

In the quantitative determinations by T.L.C., the error is upto $\pm$ 5 %.

T.L.C. was developed by Stahl[37,38] and has since been applied to the separation and analysis of lipid mixtures,[31,39] detection and evaluation of epoxy fatty acids,[35] fatty acids,[36,40] fatty acid derivatives[41] and minor components of olive oil.[42,43] Neutral lipids have been quantitatively measured by eluting and weighing,[44] methyl esters of fatty acids, oxygenated acids and mono, di and triglycerides by eluting and colorimetry of hydroxamic acid iron complexes,[36] mono, di and triglycerides by charring followed by photodensitometry(45,46)

and lipids by measuring spot areas.[30,47,48]

The significant advantages of T.L.C.[30] over existing methods of lipid (fat) analysis are:

1. Simplicity - The equipment required can be handled by inexperienced workers.
2. Speed - Complex fatty mixtures can be resolved in 5 to 30 mimtes, into different classes of compounds.
3. Efficiency - Separations are much sharper than in column or paper chromatography.
4. Sensitivity - The individual spots are more discrete and not as diffuse as in paper chromatography. Several indicators may be applied on one plate, including corrosive spray reagents.
5. Capacity - Upto 50 mg. per plate can be separated, isolated and analysed quantitatively.

Thin layer chromatography is fully exploited only when it is used in conjunction with other methods like gas-liquid, paper and/or column chromatography.

Paper chromatography

In paper chromatography, the paper (cellulose) serves as the support and is impregnated with the required stationary phase.[49] The sample to be analysed is placed near one end of this paper and this end is dipped into a suitable solvent

and allowed to remain in this position during development of the chromatogram. The solvent ascends the paper, the sample advancing also but at a slower rate. If the sample is a mixture, the individual constituents move at different rates and thus become separated into discrete spots on the paper. This is one-dimensional chromatography; two-dimensional chromatography may be employed for more complex mixtures by subsequently running a second solvent through the paper at right angles to the direction of the first chromatogram. In other modifications, the mobile phase flows downwards, horizontally or radially.[50]

After development of the chromatogram, the spots are made more visible by spraying the paper with selective indicators.[49]

For a given set of conditions, and a given substance, the ratio of the distance moved by the sample to the x distance moved by the solvent front is constant and is called the Rf value. This is a useful qualitative guide but for definite information and identification of the individual components contained in the various spots, samples of known composition or standards are run side by side with the unknown sample.

Quantitative estimates of mixtures are made by paper chromatography in several ways. For details see Block.[51]

These include

1. Visual comparison of the intensity of colour and the size of the spot
2. Cutting out the spots and eluting the substance from each of the spots
3. Measurement of the area of the spot
4. Colorimetry
5. Spot-density measurement[52] and
6. Polarography of the metal ion eluted from the spot.[52]

Kaufmann,[52,53] Mangold,[54] and Schlenk[55] have described methods for the separation and estimation of fatty acids. Methods of modifying the fatty acids, so that acids which behave similarly during chromatography, may be separated, have been devised.[56] These include hydrogenation of unsaturated acids on paper,[53] and the addition of mercury-methoxy groups to the double bonds of the esters.[57-59] The mercury-methoxy compounds, being more polar than the original esters, can be separated by partition methods. Saturated fatty acids of 20 to 30 carbon atoms have been analysed on paper as the mercuryacetate addition compounds of their allyl esters,[60] and small amounts of fats have been saponified on paper prior to paper chromatography of their fatty acids.[61] Ballance and Cromble[62] separated 40 pure fatty acids on several reversed-phase paper chromatographic

systems and have discussed the relation between structure and Rf values. They have made the method quantitative by photometric estimation of copper(as the dithiooxamide complex) retained in the paper by the copper soaps of the fatty acids. In a recent paper,[65] Kaufmann and Guldenpfennig report the analysis of fatty and resin acids of tall oil.

There are several pitfalls in paper chromatography[49] and these are

1. Correct identification of spots. A single spot does not necessarily indicate a single substance.
2. Correct use of colour tests. Even major components may be missed by improper staining.
3. Autoxidation. More than one spot may be observed when readily oxidisable samples are chromatographed.
4. Material remaining at the origin on silicic acid impregnated paper should not always be judged to be the result of autoxidation.
5. The sample analysed may contain appreciable amounts of substances which do not stain at all or stain lightly.
6. The use of strongly acidic silicic acid paper and strongly acidic solvent should be avoided.

However, paper chromatography is a convenient, quick and inexpensive method. It is a valuable tool when used in conjunction with column/gas-liquid chromatography and spectrophotometry.

Reversed-phase partition chromatography (R.P.C.)

Reversed-phase partition chromatography is column chromatography in which the stationary phase is less polar than the mobile phase.

The column contains a solid support, usually Hyflo-Supercel made non-wetting by dimethyldichlorosilane, which is coated ~~which is~~ with the stationary phase. The sample, loaded on top of the column, is developed successively with solvents of increasing polarity. The eluate is collected in small portions and titrated with methanolic alkali in presence of nitrogen, using a microburette. Titration curves are drawn by plotting the eluate fraction against the amount(ml.) of methanolic alkali required for neutralisation The position of a peak in the titration curve is characteristic for an acid or acid group in a particular solvent system and the acids present in a mixture are identified by comparing the positions of their peaks with those given by known acids in the same solvent system. By summing up the titres under each peak after allowing for the small acidity due to the eluting solvent, the results for the chromatogram are calculated.

This method was devised by Howard and Martin[64] and used for the separation and quantitation of saturated fatty

acids having 12 to 18 carbon atoms, and mixtures of saturated, unsaturated and sustituted acids. They studied the effect of lowering the percentage of water in the developing solvent, on the rates of movements of the acids and found that the more water there is present, the more slowly the acid travels down the column. They observed that using paraffin as stationary phase and 65 %. (v/v) aqueous acetone as mobile phase, oleic, elaidic and palmitic acids all travel at similar rates. Acids differing only in the extent of unsaturation are separable and hydroxy acids can be separated from non-hydroxy acids. They further suggest that saturated and unsaturated acids which run together could be readily separated after oxidation of the unsaturated components.

Silk and Hahn[65] extended this method to cover the resolution of mixtures of even numbered fatty acids from C_{16} to C24 and have shown that the method is semi-quantitaive as well as qualitative and that the quantitaive accuracy of this method is of the order of $\pm$ 10 %.

Popjak and Tietz[66] analysed fatty acids before and after hydrogenation of the unsaturated acids and extended the method(of Howard and Martin) to include C_{10} to C_{24} acids.

Savary and Dasmuelle[67] investigated the behaviour of oleic and linoleic acids and showed that these acids are separable. According to these authors, linoleic acid is

eluted rather more rapidly than myristic acid, and oleic acid at the same rate as palmitic acid. They separated these saturated and unsaturated acids after hydroxylation, by rechromatography on columns in which castor oil is the stationary phase.

Crombie, Comber and Boatman[68] investigated a large number of unsaturated acids on paraffin columns[64] and drew a number of conclusions regarding the relationship between structure and elution rate.

1. A difference of two CH_2 units in a straight chain even numbered saturated fatty acid is sufficient for complete separation from its homologues.

2. The presence of a double bond in the fatty acid chain increases the elution rate to a greater or less extent according to its position. Oleic acid is eluted with palmitic acid and these two acids are inseparable by this method (a critical pair).

3. A second double bond tends to further increase in elution rate, e.g., linoleic acid moves slightly more slowly than myristic acid although inseparable from it in all proportions.

4. The presence of a third double bond results in further increase in elution speed. Linolenic and elaeostearic acids move faster than myristic acid.

5. An acetylenic link causes a very marked change in elution

rate. One triple bond has a greater effect on elution rate than two double bonds. A second acetylenic linkage as in erythrogenic acid causes very rapid elution.

6. Ketoacids are completely separable from the unsubstituted trienoic acids.

7. The presence of hydroxy groupings in a fatty acid causes very rapid elution. The presence of two hydroxy groups appears to reduce the solubility of the acids in paraffin so much that bad tailing results. The occurrence of significant quantities of such acids in a mixture would prevent successful chromatographic analysis.

They also described a method for the analysis of milligram quantities of fatty acid mixtures containing saturated and unsaturated acids. The unsaturated acids like the oleic and linoleic acids are separable from each other but not from certain saturated fatty acids (critical pairs) which are eluted at similar rates. The saturated acids are estimated by a second chromatogram following oxidative cleavage by alkaline permanganate which removes the unsaturated acids. The amounts of unsaturated acids are then determined by difference.

Garton and Lough[69] separated all the odd numbered normal fatty acids C_9-C_{19} both from each other and from the even numbered normal fatty acids C_{18}-C_{20}. They applied this

method to the non-volatile saturated fatty acids of bovine milk fat and found that the results from R.P.C. compared well with those obtained by ester fractionation analysis of the same fatty acid mixture.

Lough and Garton[70] analysed the fatty acids of plasma lipids by applying the hydrogenation procedure of Popjak and Tietz.[66]

Gunstone and Sykes[71] developed a method which is an improvement on the earlier methods with the following advantages.

1. The results follow from chromatographic data alone, although other determinations can be used to check the accuracy of the results.
2. The unsatisfactory permanganate oxidation is replaced by ozonolysis and
3. Certain difficulties associated with the quantitative recovery of eluted acids as required in other methods using R.P.C. are avoided.

They extended the method to the quantitative estimation of epoxy and other oxygenated acids and applied it to the analysis of the component acids of some seed oils.[72]

Brenner and Mercuri[73] used a continually changing eluting system and suggest the possibility of making this method fully automatic with the development of a special

fatty acid detector.

The method is sensitive and versatile, but laborious as compared to G.L.C, T.L.C., and paper chromatography. Milligram quantities of complex mixtures containing saturated, unsaturated and oxygenated acids can be separated, identified and estimated.

Gas-liquid chromatography (G.L.C.)

Gas-liquid chromatography introduced by James and Martin[74] provides one of the most precise and rapid means of estimating fatty acids. In this method, methyl esters of fatty acids are employed for their separation because of their lower boiling ponts. The esters of the sample are made to pass through a heated column by means of an inert carrier gas such as argon, helium or nitrogen and the components of the sample are eluted with the gas and detected and measured at the exit end of the column.

The column is usually packed with an inert diatomaceous earth like Kiesulguhr, previously coated with a known amount of a non-polar stationary phase such as paraffin greases(Apiezon), or with silicone products, or with the more polar polyesters. Materials volatile at column temperatures are distributed between a moving gas phase

and a stationary liquid phase. Different constituents move through the column at different rates because of differences in partition coefficients. With non-polar phases, unsaturated and branched components emerge before the corresponding saturated ones, whereas with polar phases, unsaturated acids emerge after their saturated analogues.[75]

Columns of various diameters and lengths[76-78] and different detector systems[79-83] are in use.

Qualitative identification of components in a chromatogram is achieved by calculating either the relative retention time[84-86] for each eluted component or by calculating the carbon number[87] for each peak or by the use of standards.

The quantitative estimation of fatty acid esters is made by one of the following methods,[86]

1. By measuring the area under each peak,
2. By cutting out the curves and weighing the papers,
3. By triangulation, or
4. By automatic integration.

The first three methods were critically compared[85] and the results were found to be almost identical. The method of triangulation is technically simpler and more rapid than the first two methods. In the method of automatic integration, quantitative results are obtained by

direct reading of the detector response (number of divisions moved by the pointer) for each component.

Before quantitating an unknown sample, synthetic mixtures should be analysed and the detector response[89] to each functional group determined.

The esters of the unknown sample should be chromatographed twice, before and after hydrogenation and if necessary, on two stationary phases in order to separate and estimate the individual saturated and unsaturated esters.

The mean values from several determinations should be taken for calculation after allowing for the detector response to each functional group.

The quantitative analysis of mixtures of esters has been reported by Craig and Murty[90,91](vegetable oils), Hopkins and Chisholm[92,93](seed oils), Morris, Holman and Fontell[94](epoxy acids), Cason and Tavs[95](fatty acids), Herb, Magidman and Riemenschneider[96]; Schlenk, Mangold, Gellerman, Link, Morrissette, Holman and Hayes[97](comparison of G.L.C. results with those from U.V.), Kauffman and Lee[98] (octadecenoic acids), Kauffman, Weiss, Lee and Rockwood[99] (comparison of G.L.C. and U.V. results of oils before and after hydrogenation), Hewett, Kipping and Jaffery[100](fatty acids of montan wax), Klenk and Brucker-Voigt[101](fatty acids

of herring oil) and Caroglio and Boddi Ciammardi[102](fatty acids of olive oils).

Chromatography of all types is by far the most popular analytical tool today. In all types of column chromatography, only the eluent is analysed and substances remaining on the column are not detected. In paper and thin layer chromatography ("open columns"),[31] all components can be visualised and measured. Gas-liquid chromatography is more rapid and reproducible and has greater resolving power than the other methods.

By judicious combination of one or more of these methods, it is possible to characterise and estimate the components in a complex fatty acid mixture in the matter of a few hours. These methods are precise and rapid and milligram quantities of fatty acids can be separated and measured most conclusively. Except for gas-liquid chromatography, all the methods described are inexpensive to operate.

References

1. Insull, W. Jr., and Ahrens, E.H. Jr, Biochem. J., 1959, 72, 27.
2. Ahrens, E.H. Jr., W. Insull, Jr., J. Hirsch, W. Stoffel, M.L. Peterson, J.W. Farquhar, T. Miller and Thomasson, H.J., Lancet, 1959, 1, 115.
3. Gunstone, F.D.," An Introduction to the Chemistry of Fats and Fatty Acids," p. 42 Chapman and Hall, 1958.
4. Metcalfe, L.D., Anal. Chem., 1961, 33, 1559.
5. Holman, R.T.," Progress in the Chemistry of Fats and other Lipids", p. 104, Pergamon Press, Vol. I. 1952.
6. Claesson, S., Nature, 1947, 159, 708.
7. Stein, W.H., and Moore, S., J. Biol. Chem., 1948, 176, 337.
8. Dutton, H.J., J. Phys. Chem., 1944, 48, 179.
9. Tiselius, A., and Claesson, S., Arkiv for Kemi Min. Geol., 1942, 15B, 18.
10. Holman, R.T., and Nagdahl, L., Anal. Chem., 1951, 23, 794.
11. Lederer, E., and Lederer, M.," Chromatography," Elsevier Press, 2nd. Ed. 1957.
12. Cassidy, H.G., J. Am. Chem. Soc., 1940, 62, 3075, 3076.
13. Cassidy, H.G., J. Am. Chem. Soc., 1941, 63, 2735.
14. Nestler, F.H.N., and Cassidy, H.G., J. Am. Chem. Soc., 1950, 72, 681.
15. Graff, M.M., and Skan, E.L., Ind. Eng. Chem., Anal. Ed., 1943, 15, 340.
16. [illegible]

16. Kirchner, J.G., Prater, A.N., and Haagen-Smit, A.J., Ind. Eng. Chem., Anal. Ed., 1946, 18, 31.

17. Manunta, C., Helv. Chim. Acta, 1939, 22, 1156.

18. Majumdar, B.K., and Goswami, M.N., Indian Soap J., 1947, 12, 227.

19. Dutton, H.J., and Reinbold, C.L., J. Am. Oil Chem. Soc., 1948, 25, 120.

20. Kaufmann, H.P., and Wolf, W., Fette und Seifen, 1943, 50, 519.

21. Swift, C.E., Rose, W.G., and Jamieson, G.S., Oil and Soap, 1943, 20, 249.

22. Riemenschneider, R.W., Herb, S.F., and Nichols, P.L., J. Am. Oil Chem. Soc., 1949, 26, 371.

23. Herb, S.F., Riemenschneider, R.W., and Donaldson, J., J. Am. Oil Chem. Soc., 1951, 28, 505.

24. Lemon, H.W., Can. J. Res., 1949, 27B, 605.

25. Atherton, D., and Hilditch, T.P., J. Chem. Soc., 1944, 105.

26. Bergstrom, S., Nature, 1945, 156, 717.

27. Dugan, L.R., Beadle, B.W., and Henick, A.S., J. Am. Oil Chem. Soc., 1948, 25, 153.

28. Schuette, H.A., and Dal Nogare, S., J. Am. Oil Chem. Soc., 1951, 28, 229.

29. Morris, L.J., Hayes, H., and Holman, R.T., J. Am. Oil Chem. Soc., 1961, 38, 518.

30. Mangold, H.K., J. Am. Oil Chem. Soc., 1961, 38, 708. -Review

31. Malins, D.C., and Mangold, H.K., J. Am. Oil Chem. Soc., 1960, 37, 576.

32. Kaufmann, H.P., and Makus, Z., Fette Seifen Anstrichmittel, 1960, 62, 1014.

33. Kaufmann, H.P., Z. Makus., and Deicke, P., Fette Seifen Anstrichmittel, 1961, 63, 235.

34. Winterstein, A., Studer, A., and Ruegg, R., Chem. Ber., 1960, 93, 2951.

35. Morris, L.J., R.T. Holman and K. Fontell, J. Lipid Research, 1960, 1, 412.

36. Vioque, E., and Holman, R.T., J. Am. Oil Chem. Soc., 1962, 39, 63.

37. Stahl, E., Pharmazie, 1956, 11, 633.

38. Stahl, E., Chemiker Ztg., 1958, 82, 323.

39. Mangold, H.K., and Malins, D.C., J. Am. Oil Chem. Soc., 1960, 37, 383.

40. Mangold, H.K., and Kammerek, R., Chem. and Ind., 1961, 27, 1032.

41. Applewhite, T.H., M.J. Diamond, and Goldblatt, L.A., J. Am. Oil Chem. Soc., 1961, 38, 609.

42. Vioque, E., and Morris, L.J., J. Am. Oil Chem. Soc., 1961, 38, 485.

43. Vioque, E., L.J. Morris, and Holman, R.T., J. Am. Oil Chem. Soc., 1961, 38, 489.

44. Williams, J.A., Sharma, A., L.J. Morris, and Holman, R.T., Proc. Soc. Exptl. Biol. Med., 1960, 105, 192.

45. Privett, O.S., Black, M.L., and Lundberg, W.O., J. Am. Oil Chem. Soc., 1961, 38, 312.

46. Privett, O.S., and Blank, M.L., J.Lipid Research, 1961, 2, 37.

47. Seher, A., Nahrung, 1960, 4, 466.

48. Seher, A., Microchim. Acta, 1961, 508.

49. Rouser, G., A.J. Bauman, Nicholaides, N., and Heller, D., J. Am. Oil Chem. Soc., 1961, 38, 565. - Review

50. Mehlenbacher, V.C., J. Am. Oil Chem. Soc., 1960, 37, 613.

51. Block, R.J., " Paper Chromatography," pp. 33-50, 92-100, Academic Press. 1952.

52. Kaufmann, H.P., and Nitsch, W.H., Fette Seifen Anstrichmittel, 1954, 56, 154.

53. Kaufmann, H.P., " Analyse der Fette und Fett producte," Berlin, Springer-Verlag. 1958.

54. Mangold, H.K., Lamp, B.G., and Schlenk, H., J. Am. Chem. Soc., 1955, 77, 6070.

55. Schlenk, H., J.L. Gellerman, J.A. Tillotson, and Mangold, H.K., J.AM. Oil Chem. Soc., 1957, 34, 377.

56. Mangold, H.K., J.L. Gellerman, and Schlenk, H., Federation Proc., 1958, 17, 268.

57. Jantzen, E., and Andreas, H., Angew Chem., 1958, 70, 656.

58. Jantzen, E., and Andreas, H., Chem. Ber., 1959, 92, 1427.

59. Inouye, Y., M. Noda, and Hirayama, O., J. Am. Oil Chem. Soc., 1955, 32, 132.

60. Kaufmann, H.P., and Pollerberg, J., Fette Seifen Anstrich-mittel, 1957, 59, 815.

61. Kaufmann, H.P., and Makus, Z., Physiol. Chem., Hoppe-Seyler's, 1959, 317, 39.

62. Ballance, P.E., and Crombie, W.M.L., Biochem. J., 1958, 69, 632.

63. Kaufmann, H.P., and Guldenpfennig, R., Fette Seifen Anstrichmittel, 1961, 63, 353.

64. Howard, G.A., and Martin, A.J.P., Biochem. J., 1950, 46, 532.

65. Silk, M.H., and Hahn, H.H., Biochem. J., 1954, 56, 406.

66. Popjak, G., and Tietz, A., Biochem. J., 1954, 56, 46.

67. Savary, P., and Dasmuelle, P., Bull. Soc. Chim. Fr., 1953, 1953, 939.

68. Crombie, W.M.L., R. Comber, and Boatman, S.G., Biochem, J., 1955, 59, 309.

69. Garton, G.A., and Lough, A.K., Biochem. et Biophys. Acta, 1957, 23, 192.

70. Lough, A.K., and Garton, G.A., Biochem. J., 1957, 67, 345.

71. Gunstone, F.D., and Sykes, P.J., ~~J. Sci. Fd. Agric.~~ J. Chem. Soc., 1960, 5050.

72. Gunstone, F.D., and Sykes, P.J., J. Sci. Fd. Agric., 1961, 12, 115.

73. Brenner, R.R., and Mercuri, O., An. Asoc. quim. argent., 1959, 4, 318.

74. James, A.T., and Martin, A.J.P., Biochem. J., 1952, 50, 679.

75. Fontell, K., Holman, R.T., and Lambertsen, G., J. Lipid Research, 1960, 1, 391.

76. Lipsky, S.R., J.E. Lovelock, and Landowne, R.A., J. Am. Chem. Soc. 1959, 81, 1010.

77. Lipsky, S.R., R.A. Landowne, and Lovelock, J.E., Anal. Chem., 1959, 31, 852.

78. Beerthuis, R.K., G. Dijkstra, J.G. Kepplor, and Recourt, J.H., Ann. N.Y. Acad. Sci., 1959, 72, 616.

79. Ray, N.H., J. Appl. Chem., 1954, 4, 21.

80. Davis and Howard, Chem. and Ind., 1956, 25.

81. Martin, A.J.P., and James, A.T., Biochem. J., 1956, 63, 158.

82. Lovelock, J.E., J. Chromatog., 1958, 1, 55.

83. McWilliam, I.G., J. Appl. Chem., 1959, 9, 579.

84. Peterson, M.L., and Hirsch, J., J. Lipid Research, 1959, 1, 132.

85. Farquhar, J.W., W. Insull, P. Rosen, W. Stoffel, and Ahrens, E.H., Nutrition Reviews, 1959, 17, 1-30. (supplement)

86. James, A.T., in " Methods of Biochemical Analysis," p. 59 Vol. 8, Interscience Publishers, 1960.

87. Woodford, F.P., and van Gent, C.M., J. Lipid Research, 1960, 1, 188.

88. James, A.T., and Wheatley, V.R., Biochem. J., 1956, 63, 269.

89. Evans, R.S., and Scott, P.G.W., Nature, 1961, 190, 710.

90. Craig, B.M., and Murty, N.L., Can. J. Chem., 1958, 36, 1297.

91. Craig, B.M., and Murty, N.L., J. Am. Oil Chem. Soc., 1959, 36, 549.

92. Hopkins, C.Y., and Chisholm, M.L., Can. J. Chem., 1958, 36, 1557.

95. Hopkins, C.Y., and Chisholm, M.L., J. Am. Oil Chem. Soc., 1959, 36, 210.

94. Morris, L.J., Holman, R.T., and Fontell, K., J. Am. Oil Chem. Soc., 1960, 37, 323.

95. Cason, J., and Tavs, P., J. Biol. Chem., 1959, 234, 1401.

96. Herb, S.F., P. Magidman, and Riemenschneider, R.W., J. Am. Oil Chem. Soc., 1960, 37, 127.

97. Schlenk, H., H.K. Mangold, J.L. Gellerman, W.E. Link, R.A. Morrissette, R.T. Holman, and Hayes, N., J. Am. Oil Chem. Soc., 1960, 37, 547.

98. Kauffman, F.L., and Lee, G.D., J. Am. Oil Chem. Soc., 1960, 37, 385.

99. Kauffman, F.L., T.J. Weiss, G.D. Lee, and Rockwood, B.N., J. Am. Oil Chem. Soc., 1961, 38, 495.

100. Hewett, D.R., P.J. Kipping, and Jaffery, F.G., Nature, 1961, 192, 65.

101. Klenk, E., and Brucker-Voigt, L., Z. Physiol. Chem., 1961, 324, 1.

102. Garoglio, P.G., and Boddi Giannardi, G., Olearia, 1961, 15, 189.

This thesis describes the quantitative examination, by chromatographic procedures, of a number of seed oils. These were selected because of the possibility that they might contain acids of novel structure. However, no new acids were encountered, apart from some present in boleko oil, which are being examined by another investigator.

The general methods used in these investigations are described next and then follows an account of each of the seed oils examined.

Experimental Procedure

1. Seed oil extraction.

The seeds are separated from the husk and the kernels are crushed and extracted continuously in a Soxhlet apparatus with light petroleum(b.p.40-60) for up to 12 hours. The solvent is removed from the extract, the last traces under reduced pressure. The residual kernels are crushed again and re-extracted with a fresh amount of petroleum(b.p.40-60). This process is continued till no more oil is found in the petrol extract. The oil is then stored in a refrigerator.

2. Isolation of mixed acids free from unsaponifiable matter.

In order not to hydrolyse epoxy groups, ester-hydrolysis is effected at room temperature and particular care is taken to control the pH of the solution when liberating the acids.[1]

The oil is hydrolysed at room temperature for 24 hours with normal alcoholic potassium hydroxide solution (25 ml. for 2.0g. of the oil) and the unsaponifiable matter extracted very thoroughly.[2]

The soap solution and the washings recovered from the unsaponifiable determination, are mixed and acidified with an ion-exchange resin (ZeoCarb 225). To prevent emulsion formation, a trace of sodium acetate is added to the reaction mixture before ether extraction of the liberated acids. The ether extract is washed several times with distilled water, dried and the mixed acids recovered.

(The ion-exchange resin is recovered and, after reactivation, is used again. It is washed with acetone, and then with water, kept under concentrated hydrochloric acid for 12 hours; washed free of acid with water and stored.)

3. Hydrogenation of mixed acids.

Complete hydrogenation is effected by shaking an ethanolic solution of the mixed acids (25 mg.) with an equal amount of palladium charcoal catalyst (20 %.)[3] in an atmosphere of hydrogen for 24 hours. The catalyst is removed by centrifuging and the hydrogenated acids recovered by evaporation of the solvent.

4. Ozonolysis of mixed acids.

A sample of the mixed acids(50-100mg.), dissolved in neutral, redistilled methyl acetate, ozonised at -40° C.

(solid CO_2/acetone) with a stream of ozonised oxygen (2.5 to 3.0 %.) obtained by silent high tension electric discharge.[4] The reaction is carried out in an all glass apparatus and is complete in about 3-4 minutes. The effluent gas is bubbled through a solution of potassium iodide and starch in dilute sulphuric acid. The ozone reacts quantitatively with the unsaturated acids,[5] and the completion of the reaction is indicated by the iodide/starch indicator. The flow of ozone is continued for a time equal to that required for ozonolysis. The ozonides are decomposed according to the method of Cason and Tavs;[6] the reaction mixture is allowed to warm to room temperature and after boiling with water (ca. 5 ml.) for three hours, all the solvent is removed.

5. Reversed-phase partition chromatography (R.P.C.) procedure.

The chromatographic procedures used in the present investigation are based on the directions of Howard and Martin,[7] Silk and Hahn,[8] and Gunstone and Sykes.[1,9]

Materials and Columns

Paraffin and acetylated castor oil (A.C.O.) columns are prepared from Hyflo-Supercel, previously rendered non-

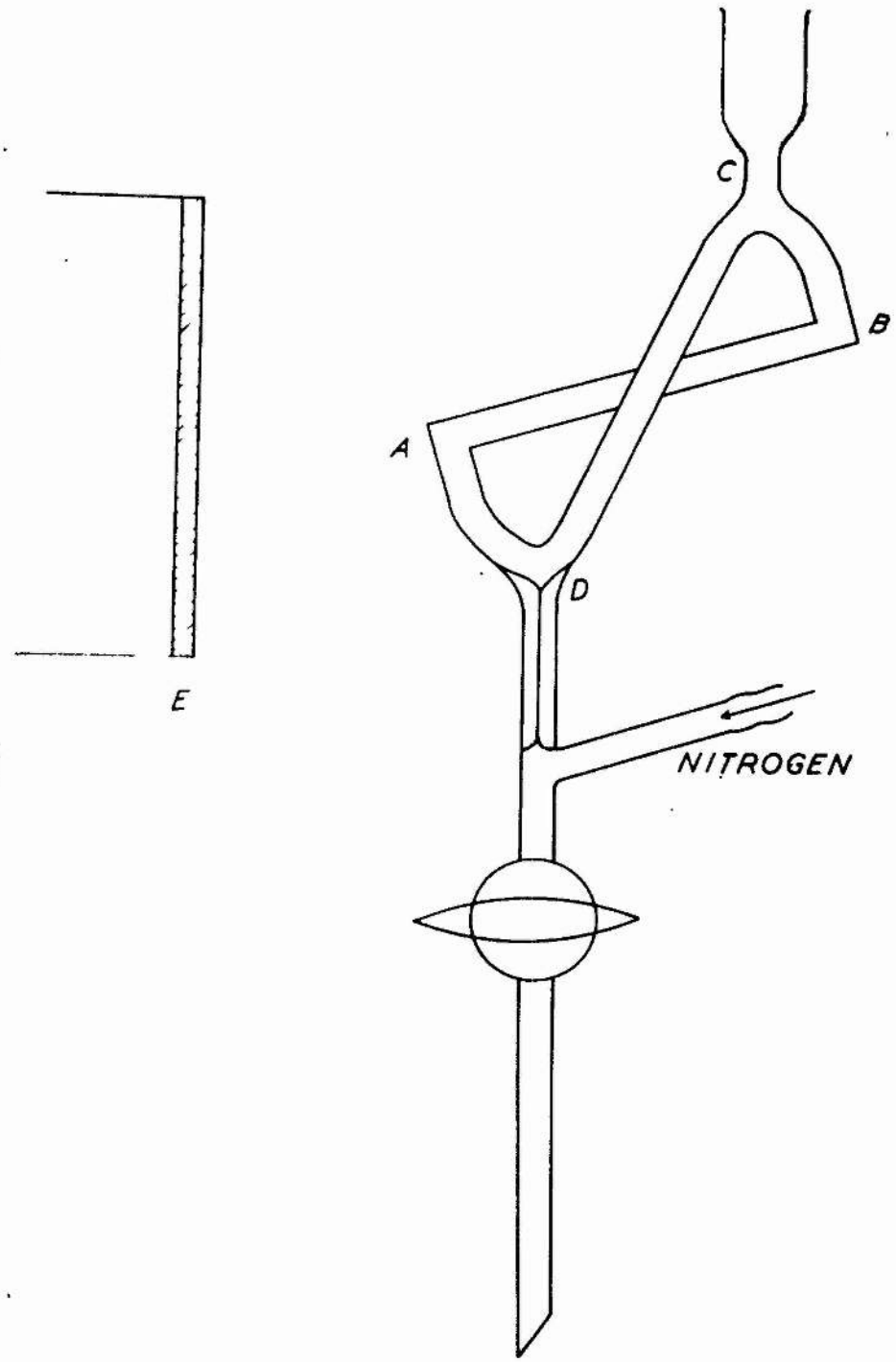


FIG 1 THE TITRATION CELL.

wetting with dimethyldichlorosilane, and loaded, developed and preserved as already described by Gunstone and Sykes.

Paraffin columns are run at 35° while the A.C.O. columns are run at room temperature. Different concentrations of aqueous acetone, equilibrated with the appropriate stationary phase and containing bromothymol blue (0.001%. w/v) as indicator, are used as the eluting solvent, after being brought to the appropriate temperature.

Chromatography

After loading the sample on top of the column, elution is carried out with a series of aqueous acetone solutions, each of which elutes a particular acid or acid group. The appropriate eluting solvents for the saturated, unsaturated and oxygenated acids etc., for both the paraffin and A.C.O. columns have been determined by Gunstone and Sykes and these are reproduced on the next page(page 33).

The eluate collects in a small siphon(2 ml.) and then passes into a specially constructed cell (see figure 1), where it is titrated, under a stream of nitrogen, with 0.01N. methanolic potassium hydroxide solution, using an Agla-micrometer syringe as a micro-burette (see figure 2). The titre for each successive fraction is tabulated serially and an eluate curve is drawn by plotting the eluate fraction against the amount(ml.) of methanolic potassium

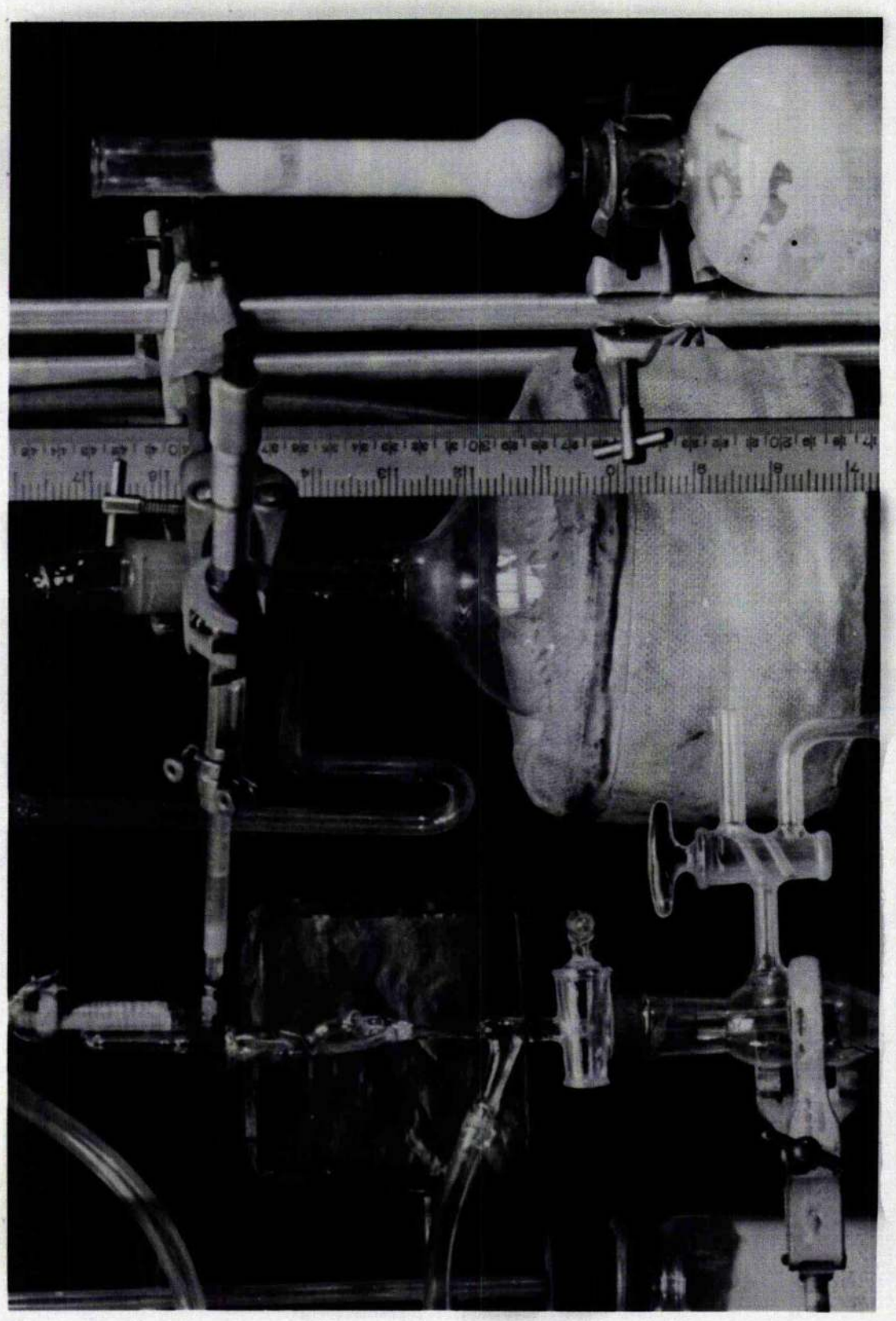


Fig. 2.

Eluting solvents. (aqueous acetone)

Kieselguhr + Paraffin Column	Acid	Kieselguhr + A.C.O. Column
43 %	Dihydroxy stearic	61 %
35 %	Hydroxy oleic	68 %
43 %	Diacetoxy stearic	74 %
53 %	Hydroxy stearic	"
"	Epoxy oleic	"
?	Diacetoxy oleic	"
58 %	Epoxy stearic	"
"	Keto stearic	"
"	Acetoxy stearic	80 %
53 %	Lauric	74 %
"	Tetradecenoic	"
"	Hexadecdienoic	"
"	Linolenic	"
62 %	Myristic	80 %
"	Hexadecenoic	"
"	Linoleic	"
67 %	Palmitic	85 %
"	Oleic	"
73 %	Stearic	"
"	Eicosenoic	"
"	Docosadienoic	"
76 %	Arachidic	
"	Docosenoic	
83 %	Behenic	

hydroxide solution required for neutralisation. Each peak in the chromatographic curve denotes the acid or acid group eluted by the particular concentration of acetone. By summing up the titres under each peak, deducting the value for the small acidity of the eluting solvent(blank), and comparing with the total titre,

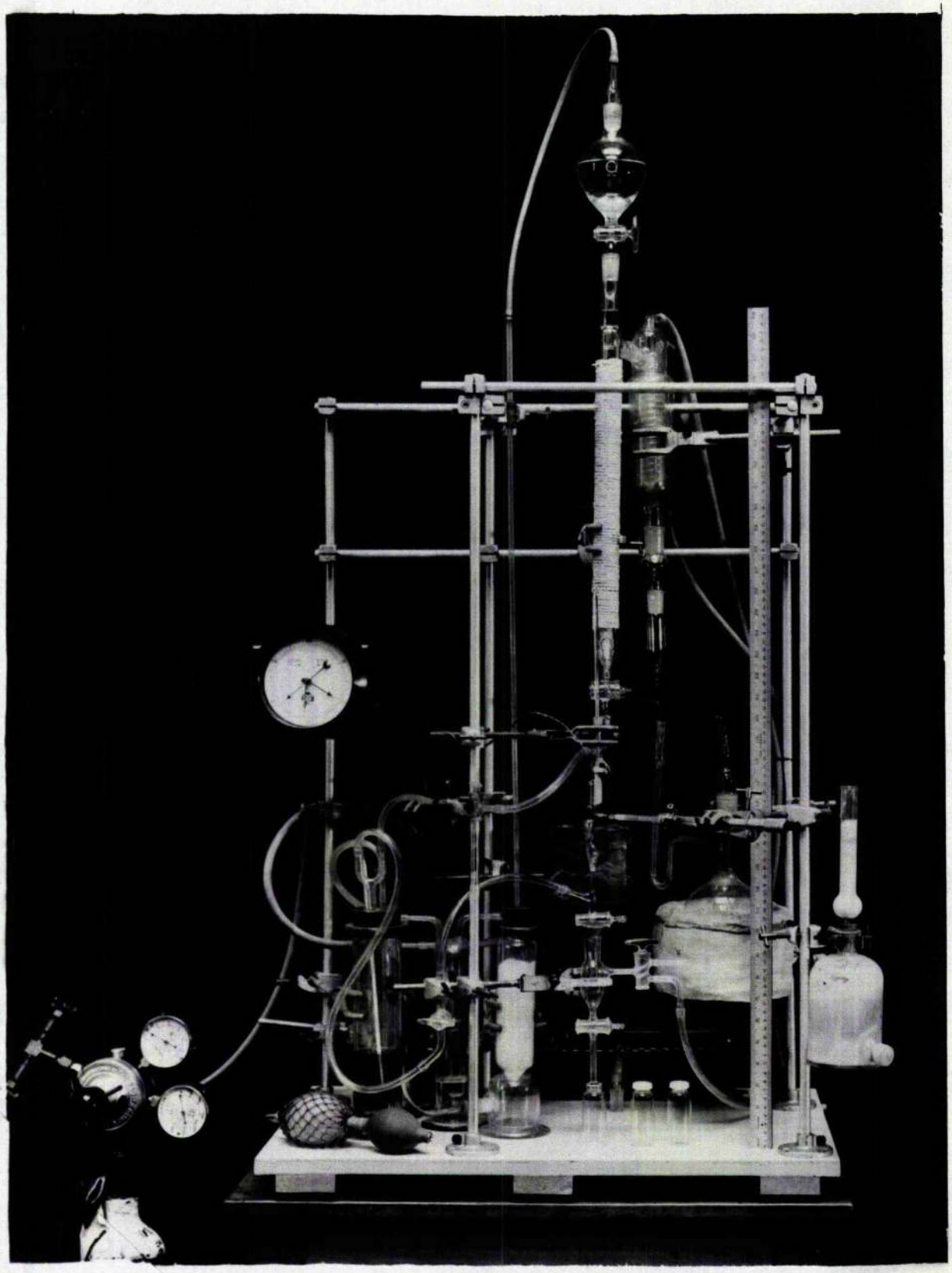


Fig. 3.

the results for the chromatogram are calculated. In this way, the mixed acids, hydrogenated mixed acids and the ozonised mixed acids are chromatographed, the elution curves drawn and the results calculated.

As a typical example and in order to illustrate the method of calculation, full details are given for the mixed acids of Jamaican Argemone mexicana seed oil. (see page 60).

6. Gas-liquid chromatography procedure.

(For details of this, see page 92)

7. Epoxide values.

(a) Morris and Holman's method.[10]

This method depends on measurement of the increased absorption in the near infra-red spectrum (at 2.795 u) which results when epoxides are converted to chlorohydrins by treatment with anhydrous ethereal hydrochloric acid solution. Morris and Holman consider the procedure to be specific for epoxides.

Conversion of epoxides to chlorohydrins.

A sample of the oil (200 mg.) is dissolved in anhydrous ether (5 ml.) and 0.2 N hydrochloric acid in an-

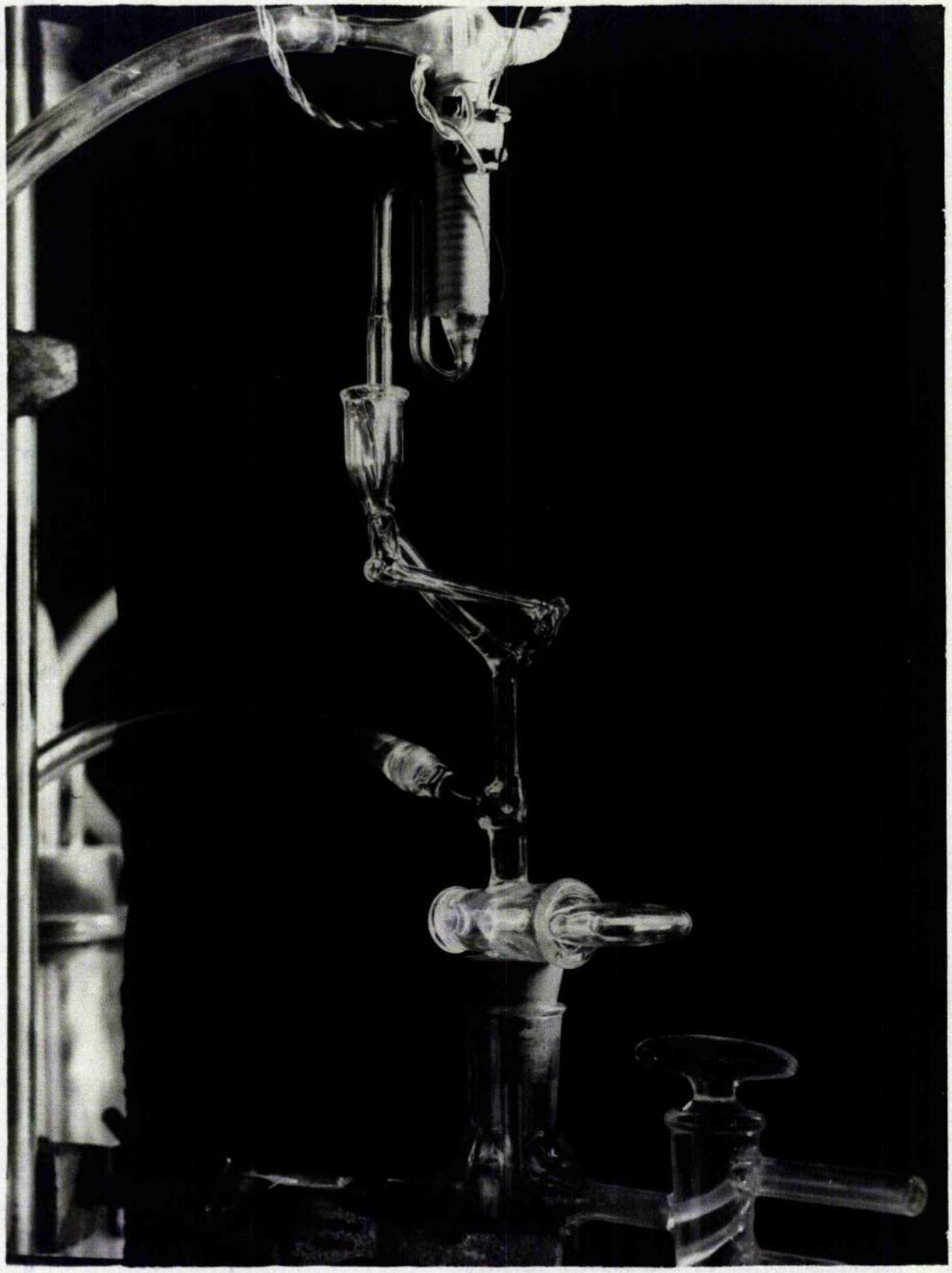


Fig. 4.

hydrous ether solution (20 ml.) is added and the mixture allowed to stand. After 5 hours, it is diluted with ether, excess hydrochloric acid removed by washing with water, dried and the product recovered by removal of the solvent under reduced pressure.

Procedure and calculation.

Spectra of the material, before and after treatment with hydrochloric acid, are recorded using solutions in dry carbon tetrachloride (3%, 1% and 0.3%) of the same concentration.

The absorbance (A) at 2.795u is measured from a base line equivalent to the absorption between 1.50 and 1.60u or from a tangent between the minima at 2.65 and 3.00u. In the present investigation, the latter method was used.

The increase in absorption ($A_t - A_u$) at 2.795u for each pair is obtained by difference and the absorptivity (a) due to chlorohydrin is calculated.

Absorbance (A) = $\log_{10} I/T$, where T=transmittance

A_t = Absorbance due to treated oil

A_u = Absorbance due to untreated oil

$A_t - A_u$ = Absorbance due to chlorohydrin

Absorptivity (a) = A/bc, where b= cell length

c= concentration

a_t= Absorptivity of treated oil

a_u= Absorptivity of untreated oil

$a_t - a_u$= Absorptivity due to chlorohydrin

The concentration of epoxides is then read off from a calibration curve prepared with known mixtures.

(Full details are given on page 119)

(b) King's method.[11]

This method is based on the reaction of epoxides with hydrochloric acid.

A solution of hydrochloric acid in dioxan is prepared by mixing 1-1.5 ml. of conc. acid with dioxan (100 ml.) which has been purified by distillation after standing with solid potassium hydroxide for several days.

About 0.2 g. of epoxide containing material is accurately weighed out in a stoppered weighing bottle of 100-150 ml. capacity and 20 ml. of the dioxan-HCl reagent is added. This is followed after 10-15 minutes by 20 ml. of neutral ethanol containing 5 drops of 1 % phenolphthalein and the solution is titrated with decinormal sodium hydroxide solution until a faint pink colour persists for 30 seconds or more. A blank determination is carried out similarly, omitting only the epoxide.

$$\% \text{ Epoxide} = \frac{(B \dagger A - T) \times M}{100 \times W}$$

$$\% \text{ Oxirane oxygen} = \frac{(B \dagger A - T) \times 0.16}{100 \times W}$$

where B, A, and T are titre values for the blank (B), a correction for any acidity in the sample (A) and for the experimental determination (T). W is the weight of the sample and M is the gram weight of pure epoxide containing 16 g. of oxirane (epoxy) oxygen.

8. Hydroxyl values.

Hydroxyl values are determined according to the method of Fritz and Schenk[12] on the methyl esters of the hydroxy acids to avoid the necessity of determining the acid values.

Reagents and solutions

1. 2 M. acetic anhydride in ethyl acetate(from a semi-microburette).
2. Distilled water (from a graduated pipette).

3. Pyridine in water (3:1) (from a graduated pipette).
4. 0.55 M. sodium hydroxide solution (titration using a semi microburette).
5. Mixed indicator solution (1 part of 0.1 % neutralised aqueous cresol red with 3 parts of 0.1 % neutralised thymol blue).

Procedure.

The methyl esters of the sample (monohydroxy-100 mg., dihydroxy-50 mg.), containing from 3 to 4 m. mols of hydroxyl is weighed accurately into a glass stoppered flask and exactly 5 ml. of 2 M. acetic anhydride in ethyl acetate are pipetted into the flask and allowed to react for not less than 5 minutes at room temperature. Then 1 to 2 ml. of water are added and the mixture shaken, followed by 10 ml. of 3:1 pyridine in water and kept for not less than 5 minutes again. This mixture is titrated against standard sodium hydroxide solution (0.55 M.) in presence of 4 drops of the mixed indicator. The colour change is from yellow to violet.

The results are expressed as % hydroxyl.

$$\% \text{ hydroxyl in sample} = \frac{17 \times (Vb - Vs) \times N}{10 \times W}$$

where Vb and Vs are the volumes of standard sodium hydroxide solution required for the blank and for the sample and W is the weight of the sample.
(The hydroxyl content of mono- and dihydroxy stearates is 5.66 and 10.7 respectively.)

9. Glycol values.[13]

The dihydroxy acid obtained from the epoxy acid is an alpha-glycol, which can be determined by periodate oxidation. This method, also, differentiates between alpha-hydroxy acids and acids containing one or more hydroxyl groups not in adjacent positions. The accuracy of the method is not greater than ± 1.5 - 2.0 %, the reproducibility of the determinations is poor[14] and erroneous results are obtained with unsaturated compounds.[15,16] Also the variation in results is greater with acids than with simple alkyl esters or triglycerides and the method cannot be used in the presence of epoxy compounds. Therefore the periodate oxidation method is not entirely suitable for the determination of the content of alpha-glycol.
Method.

The reagent is prepared by dissolving 1.4 g. of potassium periodate in 200 ml. of water and diluting to 1 litre with glacial acetic acid; stronger solutions

cannot be prepared because of the low solubility of potassium periodate. The reagent is allowed to stand for a day or two and is filtered immediately before use.

The sample, the weight of which is such that iodine liberated after oxidation is not less than 80% of that liberated in the blank, is dissolved (with warming, if necessary) in 10 ml. of a mixture of acetic acid and chloroform (2:1) contained in a glass stoppered flask. 100 ml. of the reagent are added to this solution at room temperature followed, after half an hour, by 15 ml. of 10% potassium iodide solution and 40 ml. of water. The liberated iodine is titrated with 0.1 N solution of sodium thiosulphate using starch as indicator. A blank is run at the same time but without the sample.

$$\% \text{ Glycol} = \frac{M \times (Vb - Vs) \times N}{20 \times W}$$

where M = molecular weight of glycol

N = normality of thiosulphate solution

W = weight of the sample

Vb and Vs = millilitres of thiosulphate required for the blank and for the sample respectively.

10. Characterisation of acids.

(a) Identification of di and triethenoid acids by bromination.[17]

Bromination of the unsaturated acids is carried out according to the method of Eibner and Muggenthaler.[17]

The unsaturated acids are dissolved in ethyl ether and the mixture cooled to -10°C. Liquid bromine is added drop by drop and the temperature of the acid solution is kept below -5°C. The brominated solution, on standing overnight at 0°C, deposits white crystals. The crystals are filtered and the filtrate washed with an aqueous solution of sodium thiosulphate to remove the excess of bromine. The ethereal solution is dried and the solvent removed. The yelloyish-brown viscous residue is then dissolved in petroleum ether (b.p.60-80°) and on standing overnight at 0°C., deposits crystals of insoluble tetrabromide. The crystalline bromides are then characterised by their melting points and mixed melting points with authentic samples.

Linolenic acid gives a hexabromide(m.p.181-182°) and linoleic acid a tetrabromide(m.p.113-114°).

(b) Identification of the monoethenoid acids by oxidation with 1% alkaline potassium permanganate solution.

Oxidation of the unsaturated acids is carried out according to the method of Lapworth and Mottram.[18]

The unsaturated acids (100 mg.) are warmed with an equal amount of sodium hydroxide and 6 ml. of water added and the mixture cooled; then 40 ml. of ice and water are added and the mixture well shaken. 8 ml. of 1% potassium permanganate solution are added quickly and the mixture well shaken. After 5 minutes, the mixture is decolorised by passing a current of sulphur dioxide through it, followed by the addition of about 3 ml. of concentrated hydrochloric acid. A thick white flocculent precipitate is obtained. It is drained quickly and washed with petroleum ether and dissolved in 95% alcohol. On standing overnight, white powdery crystals are deposited.

Oleic acid gives the dihydroxy stearic acid (erythro-9-10-) melting at 131-132°. Linoleic acid, if present, yields tetrahydroxy stearic acid (m.p. 166-167°).

References

1. Gunstone, F.D., & Sykes, P.J., J.Sci.Fd.Agric., 1961, 12, 115.
2. " Methods of Analysis of Oils and Fats ", B.S.684, 1958 (London, Brit.Standards Instn.)
3. Linstead, R.P., & Thomas, S.L.S., J.Chem.Soc., 1940, 1127.
4. Vogel, A.I., " Textbook of Practical Organic Chemistry, " p. 819, Longmans, 1948.
5. Boer, H., & Kooijman, E.C., Anal.chim.Acta, 1951, 5, 550.
6. Cason, J., & Tavs, P., J.Biol.Chem., 1959, 234, 1401.
7. Howard, G.A., & Martin, A.J.P., Biocem.J., 1950, 46, 532.
8. Silk, M.H., & Hahn, H.H., Biochem.J., 1954, 56, 406.
9. Gunstone, F.D., & Sykos, P.J., J.Chem.Soc., 1960, 5050.
10. Morris, L.J., & Holman, R.T., J.Lipid Research, 1961, 2, 77.
11. King, G., Nature, 1949, 164, 708; J.Chem.Soc., 1951, 1980.
12. Fritz, J.S., & Schenk, G.H., Anal.Chem., 1959, 31, 1808.
13. Bharucha, K.E., & Gunstone, F.D., J.Sci.Fd.Agric., 1955, 6, 373.
14. Hartman, J.Appl.Chem., 1955, 5, 308.
15. Kummerow & Daubert, J.Am.Oil Chem.Soc., 1950, 27, 100.
16. Parez & Malero, Grasos v.Acatites, 1955, 6, 135.
17. Eibner & Muggenthaler, (Lewkowitsch's Chemical Tchnology and Analysis of Oils and Fats, 1913, I, Ed. VI., p. 585).
18. Lapworth, A., & Mottram, E.N., J.Chem.Soc. 1925, 1628.

Holoptelea integrifolia

Seed Oil.

Holoptelea integrifolia.

Holoptelea integrifolia, a tree belonging to the family Urticaceae, is distributed fairly widely about the sub-Himalayas, Burma, Ceylon and Cochin-China. Locally (Dharwar, India) the tree is widely distributed round about Dharwar, Halyal, Dandeli and in the Kanara forest.

The bark of Holoptelea integrifolia is well-known in Ayurvedic medicine as a remedy for rheumatism. The seeds, which contain an edible fat, have a thin feathery membrane of average diameter about 1.2 inches, with the kernel at the centre.[1]

The seeds for this investigation were obtained from Dharwar (India). The oil was extracted with light petroleum and the mixed acids have been examined by reversed-phase partition chromatography as such and also after hydrogenation and after ozonolysis. The results are given on p 47-49. The monoethenoid C_{18} acid present in fractions 90-130 of the mixed acids was identified as oleic acid by conversion to erythro 9,10,dihydroxy stearic acid (m.p. and mixed m.p. 129-130°).

A survey of the literature shows that very little has been reported on the oils of the Urticaceae family. Progler[2] has given an analysis based on determination of

saturated acids and thiocyanometric data of the seed oil of Urtica dioica (stinging nettle) which suggests that it is rich in linoleic acid. Progler's figures for this oil and the present results on the seed oil of Holoptelea integrifolia are compared.

Acid(% wt)	Holoptelea integrifolia	Urtica dioica
Saturated	45	7
Hexadecenoic	2	0
Oleic	53	12
Linoleic	0	79
Linolenic	0	2

Holoptelea integrifolia seed oil.

Summary of results.

Seed and oil

Average weight of whole seed	41.36 mg.
Average weight of kernel	21.68 mg.
Oil extracted with petrol-ether (b.p. 40-60°)	25.2 % (based on seeds)

Mixed acids(excluding nonsaponifiable material).

Unsaponifiable matter	2.1%
Iodine value	47.8
Saponification equivalent	275.8

Component acids.

Acid	% mol.	% wt.
Lauric	0.3	0.2
Myristic	4.1	3.5
Palmitic	37.0	35.1
Stearic	4.3	4.5
Arachidic	1.0	1.1
Behenic	0.3	0.4
Hexadecenoic	2.0	1.9
Oleic	51.0	53.3

Calculated from above results.

Iodine value	47.8
Saponification equivalent	269.9

Detailed chromatographic results.

The three elution curves are shown in figure 3.

Mixed acids (25.2mg), S.E. 275.8, Recovery 97.9%

Alkali 1.811×10^{-2} N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq. x 10^{-5}	Mol %
12:0	37-43	0.027	0.007	0.020	0.0362	0.42
14:0	59-86	0.325	0.056	0.279	0.5053	5.83
16:0	87-134	4.312	0.096	4.216	7.6352	88.09
18:0	135-147	0.244	0.059	0.205	0.3713	4.28
20:0	157-168	0.085	0.036	0.049	0.0887	1.02
22:0	179-188	0.057	0.040	0.017	0.0308	0.36

Mixed acids after hydrogenation (35.4mg), S.E. 275.8

Recovery 96.4%, Alkali 1.811×10^{-2} N.

Acid	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol %
12:0	38-53	0.071	0.016	0.055	0.0996	0.83
14:0	60-84	0.293	0.050	0.243	0.4401	3.67
16:0	85-118	3.648	0.068	3.580	4.6724	38.97
18:0	119-159	3.770	0.123	3.647	6.6047	55.08
20:0	160-173	0.110	0.042	0.068	0.1231	1.03
22:0	184-189	0.052	0.024	0.028	0.0507	0.42

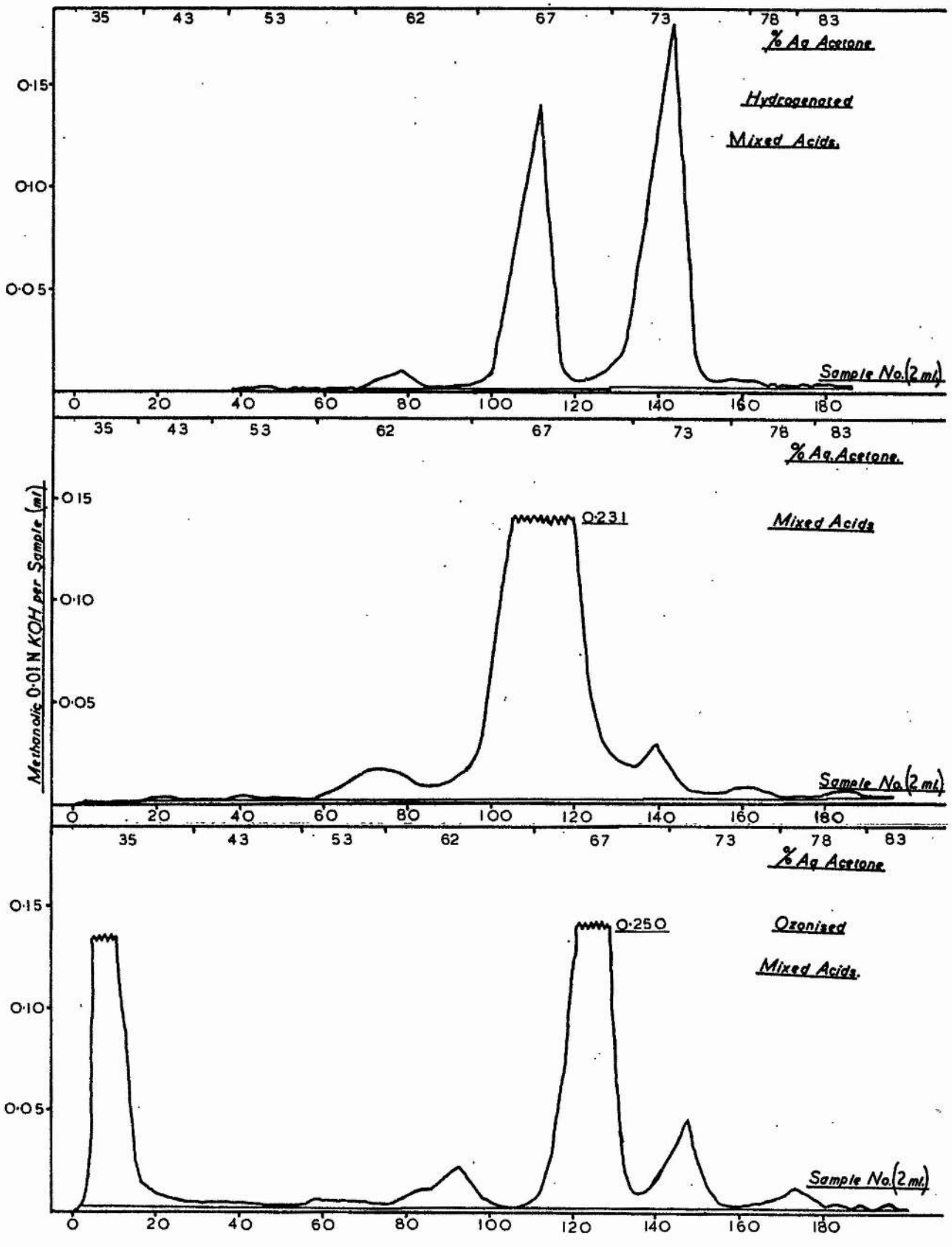


FIG 3. HOLOPTELEA INTEGRIFOLIA ACIDS.

Mixed acids after ozonolysis (32.6mg), S.E. 275.8, Recovery 48.6%., Alkali 1.811×10^{-2} N.

Acid	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq. x 10^{-5}	Mol %
12:0	56-59	0.025	0.004	0.021	0.0380	0.3
14:0	77-103	0.319	0.054	0.265	0.4799	4.1
16:0	104-135	2.481	0.064	2.417	4.3772	37.0
18:0	136-154	0.425	0.049	0.376	0.6809	5.7
20:0	162-177	0.125	0.048	0.077	0.1394	1.2
22:0	178-194	0.074	0.055	0.019	0.0344	0.3

Total alkali, alkali blank, and corrected alkali are given in millilitres.

Summary (% mols)

Acid group	12:0	14:0	16:0	18:0	20:0	22:0
Eluting solvent % (acetone)	55	62	67	73	78	83
Hydrogenated acids	0.8	3.7	39.0	55.1	1.0	0.4
Mixed acids	0.4	5.8	88.1	4.3	1.0	0.4
Ozonised acids	0.3	4.1	37.0	5.7	1.2	0.3

By solving the simultaneous equations arising from these values, the results given on pp 47 are obtained. One equation is not used and this provides a useful check on the results. The total C_{18} acids are 55.4%(calculated) and 55.1%(observed)

References

1. Badami, R.C., Proc. Indian Sci. Congr. Assoc., 1954, 41.

2. Progler, R., Fette u. Seifen, 1941, 48, 540.

Argemone mexicana

Seed Oil.

Argemone mexicana

Argemone mexicana (N.O.Papavaraceae), known as Prickly Poppy, Mexican Poppy, or Devil's Fig, is an annual indigenous to Mexico and naturalised in Brazil, India and South Africa. The small dark brown, spherical seeds are considerably larger than poppy seeds and resemble mustard seeds in appearance. The seeds yield a pale yellow clear limpid oil used in lamps and medicinally in ulcers and eruptions. The early European physicians in India used the seeds and the seed oil as a remedy for dysentry and other intestinal affections. The oil is said to have some use in India and the West Indies as an illuminant and as a purge and it is also reported to be a violent poison so that its suitability for use as an edible oil is at least questionable. Its drying properties are not such as to make it useful as a drying oil.[1,2,3]

Argemone mexicana seed oil has been the subject of several investigations. The characteristics of the oil as reported by various workers are summarised on page 53.

It is apparant that the samples examined are not very uniform. The oil content of the seed varies from 22 to 40 %, the saponification value of the oil from 183-194, and its iodine value from 107-131, though the oils which have been

Characteristics of the oil from the seeds.

Author	Saponification value	Iodine value	Acetyl value	Acid value	Unsap. matter (%)	Oil %
Bhaduri[4]	186	107	28	146	-	22
(Imp. Inst)[5]	193	124	-	22	1	40
Brambilla[6]	-	131	-	-	-	36
Iyer[7]	190	121	39	12	1	29
(Imp. Inst)[8]	192	119	-	10	2	38
Jamieson[3]	191	128	6	2	1	-
Garamendi[9]	183	127	17	3	2	38
Murari[10]	190	125	20	6	1	32
Aceiro[11]	191	124	12	11	1	30
Chakrabarty[12]	194	124	-	3	2	-

examined in detail[3,7,9-13] have a narrower range of iodine value (121-128). Acetyl values of 6-39 are also reported but in this connection it is important to recognise that acetyl values measured on natural fatty oils may arise through the presence of a hydroxy acid, but also by partial hydrolysis leading to mono and diglycerides or by autoxidation giving hydroxy or hydroperoxy compounds. There are two reports[7,13] that argemone oil contains ricinoleic acid

and other reports[3, 9-12] to the contrary. In view of current interest in natural hydroxy acids, a further examination of two samples of argemone oil has been made.

This investigation was carried out on samples of argemone oil extracted from seeds obtained from Dehra Dun, India (1955 harvest) and from Jamaica (1957 harvest) using reversed-phase partition chromatographic procedures already described. The results are summarised on pp 55 and 56 and details are given on pp 59-65

The monoethenoid C_{18} acid present in fractions 110-135 and 100-120 of the mixed acids (Jamaican oil and Indian oil respectively) was identified as oleic acid by conversion to erythro 9,10,dihydroxy stearic acid(m.p. and mixed m.p. 129-130°). The diethenoid C18 acid was isolated from fractions 65-100 and 70-95, of the mixed acids (Jamaican oil and India oil respectively) and converted to 9:10,12:13 tetrabromo stearic acid(m.p. and mixed m.p.113-114°) and identified as linoleic acid.

Argemone mexicana seed oil — Summary of results

	Jamaica	India
Origin of seeds		
Oil in seeds (%)	39.5	37.3
Unsaponifiable (%)	2.0	1.4
Iodine value of mixed acids	124.0	122.0
Saponification equivalent of mixed acids	284.0	284.8

Component acids

Source	Jamaica		India	
Acid	Mol%.	Wt.%.	Mol%.	Wt%.
Lauric	-	-	0.8	0.6
Myristic	-	-	0.4	0.3
Palmitic	10.1	9.3	12.9	12.0
Stearic	4.5	4.6	4.5	4.6
Arachidic	-	-	0.6	0.7
Behenic	-	-	0.3	0.4
Oleic	32.2	32.6	22.7	23.2
Linoleic	53.2	53.5	57.5	58.2
Calculated from above results,				
Iodine value	126.1		126.1	
Saponification equivalent	278.8		276.7	

Further examination for the presence of oxygenated acids.

(i) To concentrate any oxygenated acid which might be present, the mixed acids from both oils were separately partitioned between aqueous methanol and light petroleum (40-60°).[14] The methanolic extracts (2.0% and 0.5% from the Indian and Jamaican oil mixed acids respectively) were examined chromatographically. (for details see page 64)

(ii) Epoxy (oxirane) values[15] were measured on both samples of oils and also on some other oils known to contain and known not to contain epoxy acids, with the following results:

Oil	% oxirane oxygen
Olive	0.01
Argemone mexicana (Indian)	0.03
Argemone mexicana (Jamaican)	0.09
Cephalacroton	3.30
Epoxystearic acid	5.20

(iii) The infrared spectra of thin films of both oils were examined but no unusual peaks were identified.

It is concluded from these experiments that unusual oxygenated acids, if present at all, cannot exceed 2.0 and 0.5% in the Indian and Jamaican oils respectively. It seems most likely that these are oxidation products of linoleic

acid, which acid is present in these oils in appreciable amounts.

Discussion

Results obtained in this investigation are compared with those obtained previously and the following comment

Component acids (wt%) of argemone oil

Source	Jamaica	Mexico	Mexico	Argentina.	←------India------→				
Ref.	Present work	(3)	(9)	(11)	Present work	(7)	(13)	(10)	(12)
Lauric	-	-		-	Tr	-	-		-
Myristic	-	Tr		1	1	-	-		-
Palmitic	9	12		7	12	8	9		13
Stearic	5	2	13	-	5	6	4	21	4
Arachidic	-	-		Tr	1	-	-		1
Behenic	-	-		-	Tr	-	-		-
Lignoceric	-	Tr		-	-	-	-		-
Hexadecenoic		1	-	2	-	6	2	-	-
Oleic	33	23	31	32	23	22	26	25	19
Linoleic	53	62	56	58	58	48	48	54	62
Linolenic	-	-	Tr	-	-	Tr	3	-	-
Ricinoleic	-	-	-	-	-	10	8	-	-

is made.

1. It seems unlikely that argemone oil contains ricinoleic

or any other hydroxy acid except perhaps in very small amount(1-2%# at most) even this may have been produced by atmospheric oxidation.

2. Argemone oil contains 14-20% of saturated acids which are almost entirely palmitic and stearic acids, the former being present in greater amount. The unsaturated acids are oleic and linoleic, though traces of hexadecenoic acid and linolenic acid may also be present. The content of oleic and linoleic acid is usually in the range 19-33%, and 48-62% respectively. Argemone mexicana belongs to the natural order Papavaraceae and its seed oil is not dissimilar to poppy seed oil (of the same natural order), except that the latter is a little more unsaturated (Saturated acids 6a 10%, oleic acid 16-30%, linoleic acid 65-72%).

3. There is a slight tendency for the Central American oils to be more unsaturated than the Indian oils and this is reflected mainly in the proportion of saturated acids which is a little greater in the Indian oils.

Argemone mexicana seed oil (Jamaican)

Detailed chromatographic results

The three elution curves are shown in figure 4.

Mixed acids (26.7mg.), S.E.284, Recovery 96.6%,
Alkali 1.5553 x 10^{-2} N.

Acid group	Fr.No.	Tot.Alk.	Blank	Alk.Corr.	Eq x 10^{-5}	Mol %.
14:0	58-105	3.207	0.096	3.111	4.8385	53.3
16:0	106-140	2.535	0.070	2.465	3.8338	42.2
18:0	141-158	0.310	0.050	0.260	0.4043	4.5

Mixed acids after hydrogenation (27.5mg.), S.E.284,
Recovery 97%, Alkali 1.5553 x 10^{-2} N.

Acids	Fr.No.	Tot.Alk.	Blank	Alk.Corr.	Eq x 10^{-5}	Mol %.
16:0	71-98	0.669	0.056	0.613	0.9534	10.1
18:0	99-145	5.566	0.133	5.433	8.4500	89.9

Mixed acids after ozonolysis (54.4mg.), S.E.284,
Recovery 13.8%, Alkali 1.5553 x 10^{-2} N.

Acids	Fr.No.	Tot.Alk.	Blank	Alk.Corr.	Eq x 10^{-5}	Mol %.
16:0	104-135	1.324	0.064	1.260	1.9597	10.2
18:0	136-159	0.500	0.064	0.436	0.6781	3.5

Total alkai, alkali blank, and corrected alkali are given in millilitres.

Summary (% mol)

Acid group	14:0	16:0	18:0	20:0	22:0
Eluting solvent(%) (acetone)	62	67	75	78	83
Hydrogenated acids	-	10.1	89.9	-	-
Mixed acids	53.3	42.2	4.5	-	-
Ozonised acids	-	10.2	3.5	-	-

Calculation of results.

I. Hydrogenated acids. Equation no.

16.0 + 16.1 = 10.1 - - - (1)

18.0 + 18.1 + 18.2 = 89.9 - - - (2)

II. Mixed acids.

14.0 + 16.1 + 18.2 = 53.3 - - - (3)

16.0 + 18.1 + 20.2 = 42.2 - - - (4)

18.0 + 20.1 + 22.2 = 4.5 - - - (5)

III. Ozonised acids.

14.0 = nil - - - (6)

16.0 = 10.2 - - - (7)

18.0 = 3.5 - - - (8)

20.0 = nil - - - (9)

22.0 = nil - - - (10)

By solving these simultaneous equations, the following results are obtained.

14.0	= 0	from equation	(6).
16.0	= 10.1 or 10.2	" "	(1) & (7).
18.0	= 4.5 or 3.5	" "	(5) & (8).
20.0	= 0	" "	(9).
22.0	= 0	" "	(10).
18.1	= 32.1 or 32.0	" "	(1,4,& 7).
18.2	= 53.3	" "	(5) & (6).

Equation (2) is not used and this provides a useful check on the results.

18.0 + 18.1 + 18.2 = 89.9 (Observed)

4.5/3.5 + 32.1 + 53.3 = 89.9/88.9 (calculated)

In the final results, the following values are taken since they provide the most consistent results.

Acid	% mol.	% wt.
Palmitic	10.1	9.5
Stearic	4.5	4.6
Oleic	32.2	32.6
Linoleic	53.2	53.5

	Calculated	Observed
Saponification equivalent	278.8	284.0
Iodine value	126.1	124.0

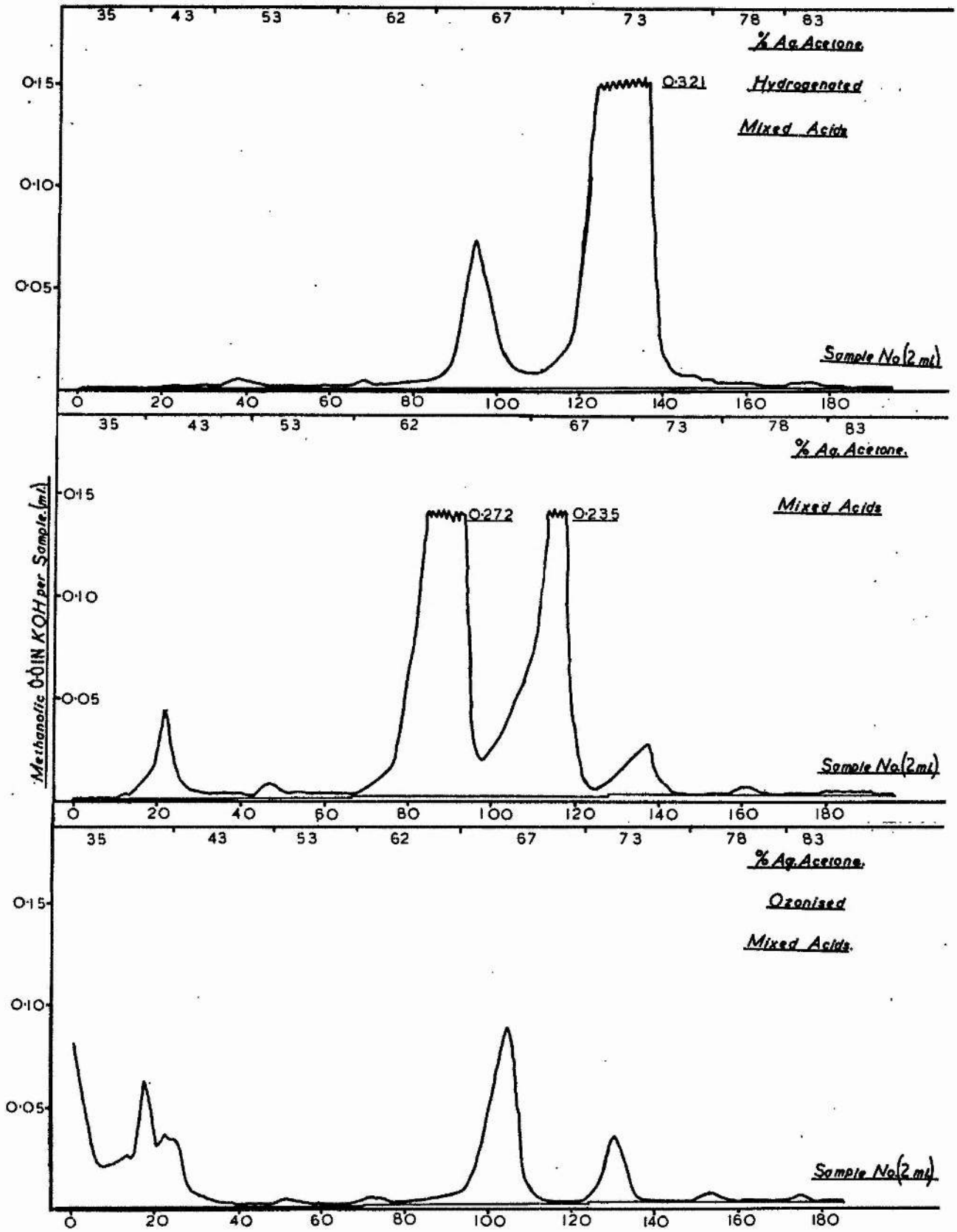


FIG 4 ARGEMONE MEXICANA ACIDS (INDIAN)

Argemone mexicana seed oil. (Indian)

Detailed chromatographic results.

The sample of the oil used was extracted in October 1957, and stored in a refrigerator. At the time of the investigation, the oil had acquired a dark brown colour.

The three elution curves are shown in figure 5.

Mixed acids (24.6mg.), S.E.284.8, Recovery 96.8%,
Alkali 1.5855 x 10^{-2} N.

Acid group	Fr.No.	Tot. Alk.	Blank	Alk.Corr.	Eq x 10^{-5}	Mol %
10:0	10-31	0.268	0.022	0.246	0.3895	4.7
12:0	42-50	0.050	0.009	0.041	0.0649	0.8
14:0	66-97	2.872	0.064	2.808	4.4464	53.2
16:0	98-124	1.934	0.054	1.880	2.9770	35.6
18:0	125-143	0.287	0.048	0.239	0.3785	4.5
20:0	153-169	0.095	0.051	0.044	0.0696	0.8
22:0	180-191	0.058	0.036	0.022	0.0348	0.4

Mixed acids after hydrogenation (25.3mg.), S.E.284.6,
Recovery 92 %, Alkali 1.5855 x 10^{-2} N.

Acid	Fr.No.	Tot.Alk.	Blank	Alk.Corr.	Eq x 10^{-5}	Mol %
12:0	31-50	0.069	0.020	0.049	0.0776	1.0
14:0	60-75	0.059	0.016	0.043	0.0681	0.8
16:0	76-106	0.717	0.052	0.665	1.0530	13.9
18:0	107-148	4.428	0.084	4.344	6.8787	84.3
20:0	149-164	0.065	0.032	0.033	0.0523	0.6
22:0	165-177	0.045	0.026	0.019	0.0301	0.4

Mixed acids after ozonolysis. (27.8mg.), S.E.284.8,
Recovery 17.8%., Alkali 1.5835 x 10^{-2} N.

Acid	Fr.No.	Tot.Alk.	Blank	Alk.Corr.	Eq x 10^{-5}	Mol %
12:0	47-59	0.050	0.013	0.037	0.0586	0.6
14:0	68-76	0.042	0.018	0.024	0.0380	0.4
16:0	80.118	0.857	0.078	0.779	1.2335	12.6
18:0	119-147	0.306	0.082	0.224	0.3547	3.6
20:0	148-161	0.064	0.042	0.022	0.0348	0.4
22:0	171-178	0.035	0.024	0.011	0.0174	0.2

Total alkali, alkali blank, and corrected alkali are given in millilitres.

Summary (mol %.)

Acid group	10:0	12:0	14:0	16:0	18:0	20:0	22:0
Eluting solvent% (acetone)	43	53	62	67	73	78	83
Hydrogenated acids	-	1.0	0.3	12.9	84.3	0.6	0.4
Mixed acids	4.7	0.8	53.2	35.6	4.5	0.8	0.4
Ozonised acids	-	0.6	0.4	12.6	3.6	0.4	0.2

These results show no unusual features except for one point which deserves comment. The mixed acids chromatogram shows the presence of an acid (4.7 % mol) eluted with 43 % acetone, while the hydrogenated acids chromatogram and the ozonised acids chromatogram do not show any acid eluting

with 43 % acetone. Therefore this acid is not saturated and since the calculation shows a discrepency of about 5 % in the C_{18} acids, the acid eluting with 43 % acetone is considered to be an oxidation product of linoleic acid and is accordingly included with this acid in the results.

The equations indicate a small amount (0.3%) of hexadecenoic acid, but this is probably experimental error and has been neglected. The stearic acid recorded by ozonolysis is 3.6%., but from the mixed acids, it is 4.5%.; the latter value is taken since it provides the most consistant results. The C_{18} acids after hydrogenation represent 84.3 %., but the sum of stearic, oleic and linoleic acids is only 80.0 %; the difference is covered by the acid eluted with 43 %. acetone (4.3 %.) which is considered to be oxidised linoleic acid. The final results are given on page 55.

Partition experiments.

The mixed acids dissolved in 500 ml. petroleum ether (b.p.40-60°) were extracted with aqueous methanol(80%.) (150 x 3). From the Indian oil(13.21g.) the methanolic extract was 0.27g.(2.0%.), from the Jamaican oil(17.7g.) it was 0.089g.(0.5%.). These small amounts were examined chromatographically. Recoveries were much lower than usual and the major component(extracted with 43% acetone) was accompanied by many other peaks.

Chromatographic results (% mol)

India oil: Hydroxy fraction(26.2mg.), Recovery 43.3 %.

Eluting solvent% (acetone)	35	43	53	62	67	73	78	83
OH-fraction	16.7	43.6	21.6	11.5	5.0	0.7	0.9	0.2

Jamaican oil: Hydroxy fraction(21.9mg.), Recovery 61.8 %.

Eluting solvent% (acetone)	35	43	53	62	67	73	78	83
OH-fraction	6.4	69.7	9.8	12.8	0.6	-	-	0.7

****

References

1. Eckey, E.W., "Vegetable Fats and Oils", p.448, Reinhold Publishing Corporation, 1954.

2. Chopra, R.N., "Indigenous Drugs of India" p.286, The Art Press, Calcutta, 1953.

3. Jamieson, G.S., and Rose, G.W., Oil and Soap, 1943, 20, 33.

4. Bhaduri, K., Am. J. Pharm., 1914, 86, 49: (C.A. 1914, 8, 1186)

5. Anon., Bull. Imp. Inst., 1922, 20, 292; (C.A. 1923, 17, 644)

6. Brambial, M., Rev. agr., 1922, 7, 56 236; Intern. Rev. Sci. Practice Agr., 1921, 1, 216 (C.A. 1923, 17, 3617)

7. Iyer, S.N., Sudborough, J.J., and Ayyer, P.R., J. Indian Inst. Sci., 1925, 8A, 29 (C.A. 1925, 19, 3607)

8. Anon, Bull. Imp. Inst., 1943, 41, 227 (C.A. 1944, 38, 4146)

9. Garamendi, M.M., and Leon, E.C., Ciencia (Mex.), 1951, 11, 212 (C.A. 1952, 46, 9260)

10. Murari, M., and Roy, A.C., J. Indian Chem. Soc., Ind. and News, Ed. 1956, 19, 177.

11. Aceiro, M.L., Bartoni, M.H., and Cattanneo, P., Anales asoc. quim. arg., 1957, 45, 59 (C.A. 1957, 51, 15971)

12. Chakrabarty, S.R., and Chakrabarty, M.M., J. Indian Chem. Soc. Ind. and News Ed., 1957, 20, 53.

13. Prakash, O., Ram, A., and Prakash, B., J. Proc. Oil Technol. Assoc., India, Kanpur, 1953, 9, 31 (C.A. 1957, 51, 13420)

14. Bharucha, K.E., and Gunstone, F.D., J. Sci. Fd. Agric., 1955, 6, 373.

15. King, G., Nature, 1949, 164, 706; J.C.S., 1951, 1980.

Boleko (Isano) Oil.

Boleko (Isano) oil.

Boleko oil, also known as Isano or Ongokea oil, is obtained from the nuts of Onguekoa Gore Englar (syn. Ongokea klaineana Pierre) of the Olacaceae family. This tree grows in the French and Belgian Congo and bears nuts, which have about 68 % kernel and 32 % shell. The kernel yields about 60 % of a light yellow fat, almost as viscous as castor oil, and about 40 % of a by-product meal which is used only as a fertilizer. The oil turns red when heated or exposed to the light and explodes when heated sufficiently. It is partly soluble in petroleum ether, ethanol and hexane, and is completely soluble in benzene, acetone, ethyl ether, carbon tetrachloride and chloroform.[1,16,25,31]

Though an extremely unsaturated oil, it does not dry when exposed in thin films, either with or without added driers, and shows very little absorption of oxygen under the same conditions.

The peculiar properties of Boleko oil are attributable to the unusual acids contained in its glycerides. It contains isanic (erythrogenic) acid, which is a highly unsaturated C_{18} acid with one terminal vinyl and two acetylenic linkings,

$CH_2{=}CH(CH_2)_4C{\equiv}CC{\equiv}C(CH_2)_7CO_2H$,

and its hydroxy derivative (isanolic acid),

$CH_2{=}CH(CH_2)_4C{\equiv}CC{\equiv}CCH(OH)(CH_2)_6CO_2H$,

together with a similar pair of acids considered to contain the chromophore $-C{\equiv}CC{\equiv}CCH{=}CH-$.[1-3,25]

The degree of unsaturation of the oil is not accurately measurable by the Wijs or Hanus iodine value methods because of the presence of conjugated triple bonds.[25] Early reports gave widely varying values (see table on next page). The hydrogenation iodine value was 316.[1] The Wijs method, with approximately three times the theoretical amount of reagent used for 24 hours, gave fairly reproducible results (250).[25]

The physical and chemical characteristics of the oil are summarised on the next page.

Boleko (isano) oil is reported to contain small amounts (2 to 4 %) of saturated and very large amounts (96 to 98 %) of unsaturated acids. These acid groups are considered separately.

Saturated acids.

Steger and van Loon[5] detected 2 % of saturated acids in the fatty acids of isano oil. Castille[7] identified caproic, caprylic, lauric, palmitic, stearic, and arachidic acids in the oil. In his account of isano oil and its

Characteristics of Boleko (Isano) oil.

	References					
	5	6	7	12	14	25
Saponification value	187	189	191	193	-	191
Acid number	21	2	4	5	-	-
Acetyl number	69	-	67	69	89	79
d_n^{20}	0.984	0.975	0.945	-	-	0.978
n_D^{20}	1.510	-	1.508	-	-	1.508
Unsaponifiable matter(%)	-	1.1	3.3	0.7	3.2	0.9
Iodine number	103-252 (a)	186 (c)	143	228	206 198	230 (f)
"	117-321 (b)	355 (d)				
"	280 (c)					
"	316 (d)					

(a) Wijs iodine value determinetion ranging from 5 minutes to 48 hours.

(b) Kaufmann iodine value determination ranging from 15 minutes to 78 hours.

(c) Iodine value of hydrogenated and acetylated oil.

(d) ~~MXJK~~ Hydrogenation iodine number.

(e) Wijs iodine value after 2 hours.

(f) Wijs iodine value with excess reagent and after 24 hrs.

use in industry, Fauve[12] reported 3.6 % saturated acids, while Kaufmann, Baltes and Herminghaus[13] detected only a small amount of these acids. Seher[17-19] isolated stearic acid from the oil. Dupont, Dulon and Pouliquen,[23] de Vries,[24] and Pouliquen[27] all detected about 3 % saturated acids in the oil.

Unsaturated acids.

This group of acids is reported to contain large amounts of erythrogenic (isanic), isanolic, bolekic and bolekoic (goric) acids, together with smaller amounts of oleic, elaidic, linoleic and linolenic acids.

Erythrogenic (isanic) acid, ($C_{18}H_{26}O_2$, m.p. 40°) is now known to have the structure

$$CH_2 = CH (CH_2)_4 C \equiv C - C \equiv C (CH_2)_7 CO_2 H$$

and is thus one of the growing number of acetylenic acids occurring naturally as triglycerides.[28,30,32-36]

Early workers, including Hebert,[4] Steger and van Loon,[5] Boekenoogen[6] and Castille,[7] isolated a highly unsaturated acid from boleko oil but were unable to agree whether or not it contained acetylenic groups, the names isanic and erythrogenic acid[7] being assigned to this comound. Castille[7] concluded that it had the structure I or II and in a series of papers Steger and van Loon[8-10] confirmed

these findings, expressed a preference for structure II and concluded that this was the main component of isano oil. Castille[11] also concluded that structure II was the

I $CH_2=CHC\equiv C(CH_2)_4C\equiv C(CH_2)_7CO_2H$

II $CH_2=CH(CH_2)_4C\equiv CC\equiv C(CH_2)_7CO_2H$

correct one and this view has been confirmed by Armitage, Cook, Entwistle, Jones and Whiting[15] who consider that the U.V. spectrum reported by Castille[7] was inconsistent with structure I. (The examination of the low intensity U.V.spectrum is complicated by the presence, in smaller proportion, of other strongly absorbing acids). Black and Weedon[16] synthesised an acid of this structure and proved it to be identical with natural erythrogenic acid. The content of this acid in isano oil has been reported as 10 % (Fauve)[12], appreciable (Kaufmann, Baltes and Herminghaus[13]), 48 % (Dupont, Dulon and Pouliquen[23]), 51 % (de Vries[24]), 46 % (Kneeland, Kyriacou and Purdy[25]), 30-40 % (Pouliquen[27]).

Isanolic acid ($C_{18}H_{36}O_3$) is considered to have the structure $CH_2=CH(CH_2)_4C\equiv CC\equiv CCH(OH)(CH_2)_6CO_2H$. The presence of a hydroxy acid in appreciable amount (44 %) was first recognised by Kaufmann, Baltes and Herminghaus[13] who showed its U.V. spectra to be similar to that of isanic

acid. They suggested the structure (III) On the basis of a wrong structure for isanic acid,

(III) $CH_3(CH_2)_2CH{=}CHC{\equiv}CC{\equiv}CCH_2CH(OH)(CH_2)_6CO_2H$.

Riley[14] concentrated the hydroxy acid in the petroleum ether insoluble fraction of the mixed acids, and after hydrogenation, acetylation, and separation by distillation, the product was shown to be 8-hydroxy stearic acid by oxidation, formation of the oxime, Beckmann rearrangement, and hydrolysis of the resulting amides.

Meade[22] isolated the hydroxy acid by phthalation and showed it to be a mixture of at least two derivatives of 8-hydroxy stearic acid, containing a diyne and enediyne chromophore.

The amount of this acid has been reported as 35 % (Meade[22]), 36 % (Dupont, Dulon and Pouliquen[23]), and 40 % (de Vries[24]), so that together with isanic acid it accounts for about 80 % of the boleko acids.

Bolekic acid is a highly unsaturated C_{18} acid (m. p. 17). Meade[22] noticed that natural isanic acid exhibits a more complex spectrum than the synthetic acid; the bands characteristic of the synthetic diyne acids are present together with others of longer wavelengths, suggesting that even after careful purification, the natural acid still contains a small amount (2-3 %) of

an impurity with an enediyne chromophore, -C≡CC≡CCH=CH-. He isolated this acid by its inability to form a urea complex at -6°C after removal of all hydroxy acids by phthalation. This acid is hydrogenated to stearic acid taking up 4.9 moles of hydrogen and shows cis-unsaturation by infra red absorption, and typical conjugated enediyne ultraviolet absorption. It gives a methyl ester and thence a beta-hydroxyethylamide, m.p. 47-48°C., which is fairly stable in air. He called this acid bolekic acid.

Experiments carried out by Mr. Sealy have shown that this acid is octadec-9,11,diyne 13 cis enoic acid i.e.,

$$CH_3(CH_2)_3CH{=}CHC{\equiv}CC{\equiv}C(CH_2)_7CO_2H \quad .$$

Dupont, Dulon and Pouliquen[23] determined 3 % of bolekic acid by chromatographing boleko oil on a silica gel column.

Bolekoic acid (goric acid). 8-hydroxy octadec-14-ene,10,12, diynoic acid.

Isano oil also contains a second hydroxy acid(4-5%)[22,23] with the same enediyne chromophore as bolekic acid for which Beher[18] has suggested the structure

IV $CH_3(CH_2)_2CH{=}CHC{\equiv}CC{\equiv}CCH_2CH(OH)(CH_2)_6CO_2H$.

This is being examined by Mr. Sealy.

Other unsaturated acids. Boleko (isano) oil is reported to contain also smaller proportions of oleic,[12]

elaidic,[17] linoleic,[12] linolenic (1.6 to 6 %)[13,17,23,24] and other acids.[24]

The composition of the oil reported by various workers is summarised on next page.

Composition of Boleko (Isano) Oil.

References	5	7	12	16	17	22	23	24	25	27
Acid %.										
Saturated	2	a*	4	S	a	-	3	3	-	3
Unsaturated	98	-	96	-	-	-	-	-	-	-
Isanic (erythrogenic)	-	a	10	A	a	35	48	51	46	-
Isanolic	-	-	-	44	a	a	36	40	44	-
Bolekic	-	-	-	-	-	a	3	-	9	-
Coric (bolekolc)	-	-	-	-	-	5	4	-	-	-
Elaidic	-	-	-	-	a	-	-	-	-	-
Linolenic	-	-	-	S	a	-	6	2	-	-
Ethylenic	-	-	-	-	-	-	-	-	-	9
Hydroxy-acetylenic	-	-	-	-	-	-	-	-	-	40-50
nonhydroxy-acetylenic	-	-	-	-	-	-	-	-	-	30-40
Others	-	-	-	-	-	-	-	4	-	-

a = acid present; but percentage not given.

S = small amount present.

A = appreciable amount present.

a* = includes caproic, caprylic, lauric, palmitic, stearic and arachidic acids.

A new investigation of Boleko (Isano) oil has recently been undertaken in this department in the hope of (a) defining more clearly the structure of polyunsaturated acids, and

(b) analysing quantitatively the component acids by chromatographic procedures.

The first part of this project has been undertaken by Mr. A.J.Sealy and will be reported elsewhere. An account of the second part now follows.

The component acids of boleko oil.

A sample of boleko oil, provided by the Paint Research Station, has been used throughout these studies.

Three related investigations have been made into the component acids of boleko oil. These give generally similar results, and are complementary to one another.

(A) The first is based on the examination of mixed acids by reversed-phase partition chromatography, before and after hydrogenation (pp 80-81).

(B) The second investigation was designed primarily to concentrate various acids for structural examination but, since it provides additional quantitative information, it is included here (pp 82-91).

(C) The third investigation is an attempt at quantitative determination of the component acids by gas-liquid chromatography of the mixed methyl esters, before and after hydrogenation (pp 92-98).

The results obtained from these studies are given on page 79.

Boleko oil. Summary of results.

Component acids.

Acid	(A) (% mol)	(B) (% mol)	(C) (% wt.)	Final results. (% wt.)
Myristic	1.4	6.4	-	1
Linoleic (a)	4.3		?	5
Palmitic	3.7	18.5	3.8	4
Oleic (b)	12.8		?	13
Stearic (c)	1.7	1.3	?	1
(a) + (b) + (c)	18.8	-	20.9	-
Ximenynic		59.2	1.1	1
Isanic	52.9		46.6	33
Bolekic		10.8		11
Hydroxy isanic	22.4	20.0	27.6	21
Hydroxy bolekic		3.6		4
C_{20} & C_{22} acids	0.8	1.1	-	1

Boleko oil Detailed results.

(A). Examination by R.P.C. of the mixed acids before and after hydrogenation.

Summary of R.P.C. results. (% mol)

Acid group	10:0	12:0	14:0	16:0	18:0	20:0	22:0
Eluting solvent (% acetone)	43	53	62	67	73	78	83
Mixed acids	23.5	51.9	5.7	16.5	1.7	0.6	1.1
Hydrogenated mixed acids.	16.0	6.2	1.4	3.7	72.7	-	-

These figures give the results on page 79 (column A). It has been assumed that lauric and hexadecenoic acids are absent. The oxygenated acids are eluted in two portions after hydrogenation (16.0 % with 43 % acetone and 6.2 % with 53 % acetone). This is discussed on page 93 .

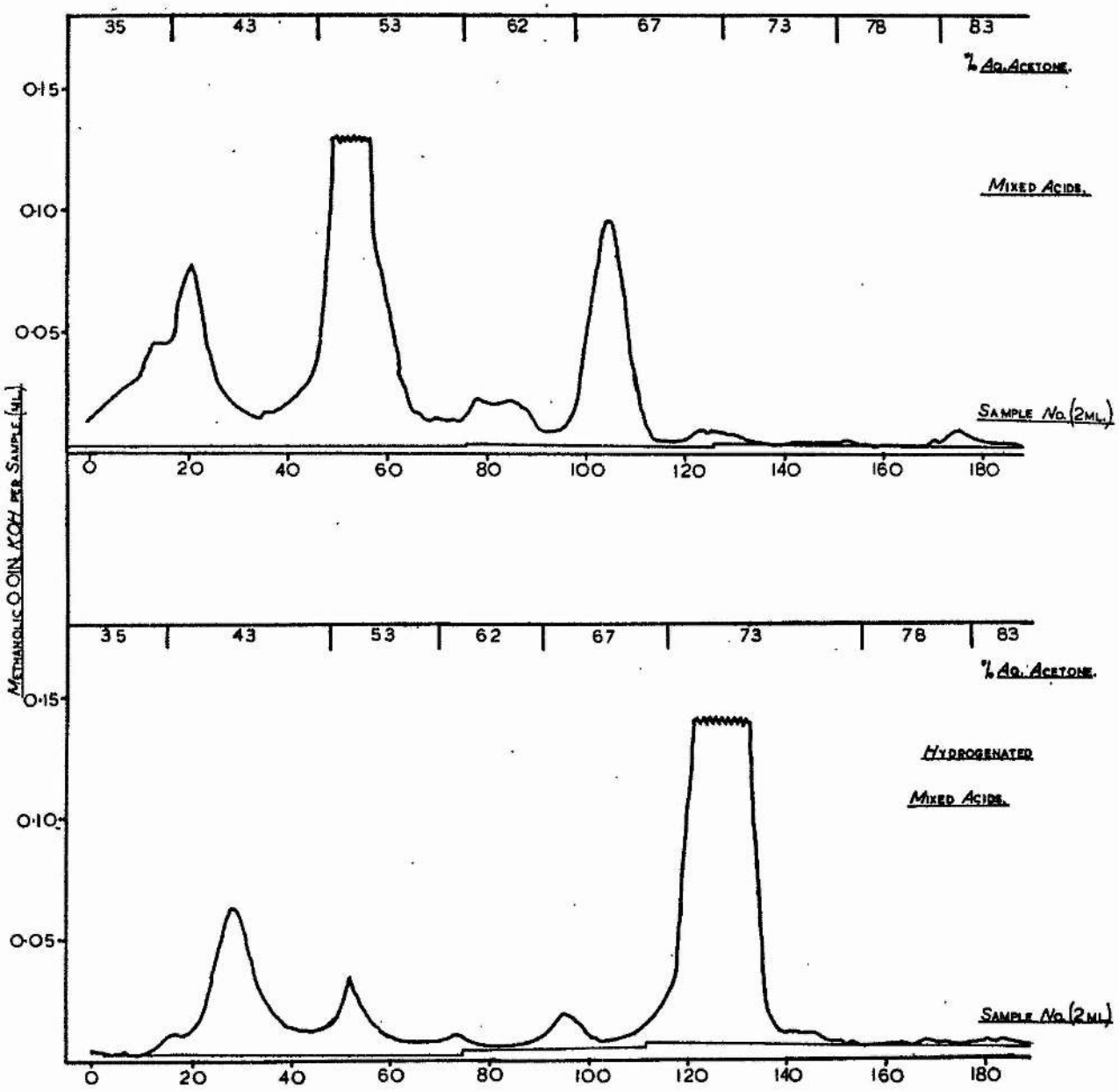


FIG. 5 Boleko (Isano) Acids.

Detailed chromatographic results.

Mixed acids (28.5mg.), S.E.317.6, Recovery 95.6%,
Alkali 1.5644 x 10^{-2} N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol%
10:0	1-34	1.266	0.034	1.232	1.9275	22.51
12:0	35-74	2.879	0.040	2.839	4.4413	51.88
14:0	75-93	0.349	0.038	0.311	0.4865	5.68
16:0	94-117	0.948	0.048	0.900	1.4079	16.45
18:0	118-137	0.146	0.050	0.096	0.1502	1.75
20:0	141-159	0.089	0.057	0.032	0.0500	0.58
22:0	168-187	0.122	0.060	0.062	0.0969	1.13

Hydrogenated mixed acids (24.5mg.), S.E.317.6,
Recovery 98.5 %., Alkali 1.5644 x 10^{-2} N.

Acid	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol%
10:0	10-43	0.813	0.034	0.779	1.2186	16.01
12:0	44-61	0.320	0.018	0.302	0.4724	6.20
14:0	70-79	0.086	0.020	0.066	0.1032	1.35
16:0	86-103	0.216	0.036	0.180	0.2816	3.70
18:0	104-152	3.672	0.134	3.538	5.5348	72.72

(B). Examination by R.P.C. after partition.[87]

The boleko mixed acids (29.0 g.), dissolved in 500 ml petroleum ether (40-60°) previously equilibrated with 80% aqueous methanol, were partitioned between petroleum ether (40-60°)(p_1 to p_4 containing 500 ml. and 3 x 200 ml. respectively), and 80% aqueous methanol, also previously equilibrated with petroleum ether (40-60°)(m_4 to m_1 containing 4 x 200 ml. respectively). The hydroxy fractions (M_2=2.0g.; M_1=7.0g.) were recovered from the methanol extract and the non-hydroxy fractions (P_1=16.8g.; P_2=3.2g.) from the petroleum ether extract.

p_1	p_2	p_3	p_4	:	m_4	m_3	m_2	m_1
500 ml.	200 ml.	200 ml.	200 ml.		200 ml.	200 ml.	200 ml.	200 ml.
P_1	P_2					M_2		M_1
16.8 g.	3.2 g.					2.0 g.		7.0 g.

Four fractions were obtained.

Main petrol solution	P_1	16.8 g.	58 %.
Minor petrol solution	P_2	3.2 g.	11 %.
Second methanol extract	M_2	2.0 g.	7 %
First methanol extract	M_1	7.0 g.	24 %
	Total	29.0 g.	

Summary of R.P.C. results (% mol)

Acid gr.	10:0	12:0	14:0	16:0	18:0	20:0	22:0	Recovery
Eluting solvent% acetone	43	53	62	67	73	78	83	%
P_1(58%)	0.9	56.5	8.5	29.9	2.3	1.0	0.9	89.9
P_2(11%)	-	88.5	4.4	7.1	-	-	-	97.3
M_2(7%)	46.2*	50.9	1.9	1.1	-	-	-	82.3
	45.1*	51.5	2.0	1.3	-	-	-	85.2
M_1(24%)	82.7*	11.8	4.0	0.5	-	-	1.0	55.0
	83.1*	11.4	3.4	2.1	-	-	-	71.3
M_1Hydr.	65.2	12.8	5.3	1.0	15.1	-	0.6	88.1
M_2Hydr.	32.4	9.5	2.5	1.2	52.2	-	2.1	82.7

* = sum of several incompletely eluted peaks.

The above results can be adjusted to allow for the amount of each fraction and then summed up for comparison with results obtained in investigation (A).

(see page 84.)

Acid group	10:0	12:0	14:0	16:0	18:0	20:0	22:0
Eluting solvent% (acetone)	43	53	62	67	73	78	83
P_1	0.5	33.9	4.9	17.3	1.3	0.6	0.5
P_2	-	9.7	0.5	0.8	-	-	-
M_2	3.5	3.6	0.1	0.1	-	-	-
	5.2	3.6	0.1	0.1	-	-	-
M_1	19.9	2.8	1.0	0.1	-	-	0.2
	19.9	2.7	0.8	0.5	-	-	-
Total	23.9	49.9	6.4	18.5	1.3	0.6	0.7
Mixed acids(A).	22.5	51.9	5.7	16.5	1.7	0.6	1.1

Comment.

1. There is good agreement of results for chromatograms run twice (M_2 and M_1).
2. Low recoveries for M fractions, particularly M_1, are probably due to the presence of nonsaponifiable material which tends to concentrate in the alcoholic extracts.
3. The results obtained after partition agree reasonably well with those obtained in the investigation (A) of mixed acids.
4. The investigation of fractions P_1 , P_2, M_2 & M_1 ,

though not primarily designed for analytical purposes, leads to the following results.

	Unsatd. OH-acids.	Highly unsatd. C_{18} acids.	C_{14} + C_{18}.	C_{16} + C_{18}.	C_{18}.	$C_{20/22}$.	Total
P_1	0.5	33.9	4.9	17.2	1.3	1.1	58.0
P_2	-	9.7	0.5	0.8	-	-	11.0
M_2	5.2	3.6	0.1	0.1	-	-	7.0
M_1	19.9	2.8	0.9	0.3	-	-	24.0
	25.6	50.0	6.4	18.4	1.3	1.1	=100.0

5. More detailed information concerning the unsaturated hydroxy acids and the highly unsaturated C_{18} acids, has been derived from measurements of the U.V. absorption spectrum of these four fractions. (These Spectroscopic measurements were made by Mr. Sealy). The results so obtained are given on page 79 (column B) and details on pp 86 - 91

Detailed chromatographic results.

P_1 acids. (22.9mg.), S.E. 290, Recovery 89.9 %.,
Alkali 1.5644 x 10^{-2} N.

Acid group	Fr.No.	Tot.Alk.	Blank	Alk.Corr.	Eq x 10^{-5}	Mol %
10:0	14-21	0.050	0.008	0.042	0.0657	0.92
12:0	36-60	2.595	0.025	2.570	4.0173	56.46
14:0	61-72	0.219	0.024	0.195	0.3050	4.28
16:0	73-86	0.220	0.028	0.192	0.3003	4.22
18:0	87-111	1.421	0.060	1.361	2.1291	29.92
18:0	112:132	0.168	0.063	0.105	0.1642	2.30
20:0	136-146	0.078	0.033	0.045	0.0703	0.98
22:0	147-157	0.073	0.033	0.040	0.0625	0.87

P_2 acids. (27.5mg.), S.E.290, Recovery 97.3 %.,
Alkali 1.5644 x 10^{-2} N.

Acid group	Fr.No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol%
12:0	55-70	5.255	0.036	5.219	8.1646	88.55
14:0	71-80	0.142	0.020	0.122	0.1908	2.07
14:0	81-94	0.161	0.028	0.133	0.2080	2.25
16:0	95-112	0.456	0.036	0.420	0.6570	7.12

M_2 acids. These were examined twice.

1) 27.3mg. S.E. 295, Recovery 82.3 %, Alkali 1.5644 x 10^{-2}N.

Acid group	Fr.No.	Tot.Alk.	Blank	Alk.Corr.	Eq x 10^{-5}	Mol%
10:0	1-10	0.381	0.010	0.371	0.580	7.61
	11-17	0.608	0.007	0.601	0.940	12.34
	18-27	0.955	0.010	0.945	1.478	19.41
	28-39	0.344	0.012	0.332	0.519	6.81
12:0	40-65	2.503	0.026	2.477	3.875	50.89
14:0	66-81	0.122	0.032	0.090	0.141	1.85
16:0	94-107	0.080	0.028	0.052	0.081	1.06

11) 23.5mg. S.E. 295, Recovery 85.2%, Alkali 1.5644 x 10^{-2}N.

Acid group	Fr.No.	Tot.Alk.	Blank	Alk.Corr.	Eq x 10^{-5}	Mol%
10:0	1-10	0.255	0.010	0.245	0.3832	5.64
	11-18	0.367	0.008	0.359	0.5616	8.27
	19-28	1.075	0.010	1.065	1.6660	24.53
	29-39	0.302	0.011	0.291	0.4552	6.70
12:0	40-69	2.267	0.030	2.237	3.4995	51.53
14:0	70:92	0.133	0.046	0.087	0.1361	2.00
16:0	93:111	0.114	0.057	0.057	0.0891	1.31

M_1 acids. These were examined twice.

i) 22.8mg. S.E.300, Recovery 55.0%, Alkali 1.5644×10^{-2} N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq $\times 10^{-5}$	Mol %
10:0	1-8	0.468	0.008	0.460	0.7196	17.25
	9-16	0.817	0.008	0.809	1.2650	30.38
	17-39	0.960	0.023	0.937	1.4660	35.11
12:0	40-61	0.338	0.022	0.316	0.4940	11.84
14:0	62-80	0.144	0.038	0.106	0.1650	3.97
16:0	93-100	0.030	0.016	0.014	0.0220	0.52
22:0	125-153	0.053	0.027	0.026	0.0400	0.97

ii) 24.7mg. S.E.300, Recovery 71.3%, Alkali 1.5644×10^{-2} N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq $\times 10^{-5}$	Mol %
10:0	1-10	0.272	0.010	0.262	0.410	6.99
	11-42	2.885	0.032	2.853	4.463	76.10
12:0	43-68	0.458	0.031	0.427	0.668	11.39
14:0	69-82	0.155	0.028	0.127	0.198	3.37
22:0	91-107	0.129	0.049	0.080	0.125	2.13

Hydrogenated M_2 acids. (24.4mg.), S.E.395, Recovery 88.1 %,

Alkali 1.5644 x 10^{-2} N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol %
10:0	19-54	1.506	0.046	1.460	2.284	52.4
12:0	55-80	0.454	0.026	0.428	0.669	9.5
14:0	84-97	0.144	0.028	0.116	0.181	2.5
16:0	102-110	0.072	0.018	0.054	0.084	1.2
18:0	117-145	2.435	0.082	2.353	3.681	52.2
22:0	146-168	0.163	0.069	0.094	0.147	2.1

Hydrogenated M_1 acids. (26.1mg.), S.E.310, Recovery 82.7 %,

Alkali 1.5644 x 10^{-2} N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol%
10:0	1-13	0.084	0.013	0.071	0.1110	1.60
	14-59	2.876	0.046	2.830	4.4272	63.56
12:0	60-83	0.593	0.024	0.569	0.8901	12.78
14:0	84-103	0.275	0.040	0.235	0.3676	5.27
16:0	108-119	0.069	0.024	0.045	0.0704	1.01
18:0	127-151	0.745	0.071	0.674	1.0544	15.14
22:0	157-167	0.061	0.033	0.028	0.0438	0.62

Spectroscopic examinations.

The $E^{1\%}_{1cm}$ value for the 6 maxima between 214 mu and 283 mu measured in methanol solution are give below.

λ max. (mu)	214	228	240	255	267	283
Mixed acids	222	60	70	71	85	69
P_1	211	52	58	55	76	60
P_2	355	78	103	112	144	116
M_2	295	61	75	85	110	89
M_1	177	73	86	73	78	64
Bolekic acid[38]	?	112	230	427	635	496

In the following calculations, the values given by Meade[38] are used and, as a first approximation, the same values are used for hydroxy bolekic acid. These values may not be very accurate but they are the best available at the present time.

When the content of bolekic and hydroxy bolekic acids is calculated from the above figures, it is apparant that acids with other chromophores are interfering with the results for the bands of lower wavelength and the value used in these calculations is the average value from the three bands of higher wavelength; thus:

Proportion of bolekic and hydroxy bolekic acid from U.V. measurements.

λ max (mu)	214	228	240	255	267	283	mean value
Mixed acids	?	54	30	17	13	14	15
P_1	?	46	25	13	12	12	12
P_2	?	70	49	26	22	24	24
M_2	?	55	33	20	17	18	18
M_1	?	65	37	17	12	13	14

These results are considered in conjuction with those on page 85.

P_1 contains 7.0% of bolekic acid (12 % of 58.0). neglecting the small amount of unsaturated hydroxy acids in this fraction. P_2 similarly contains 2.6 % (24 % of 11.0) of bolekic acid.

In the M fractions, it is assumed that bolekic and hydroxy bolekic acids are present in the same ratio as the unsaturated hydroxy acids and highly unsaturated C_{18} acids; i.e., 3.6 : 3.2 and 2.8 : 19.9 in M_1.

This gives the figures on page 79 (column B).

(C). Examination of boleko acids by gas-liquid chromatography. (G.L.C.)

Qualitative examination.

The methyl esters of the mixed acids of boleko oil were examined by G.L.C. before and after hydrogenation and the carbon numbers[59] calculated.

The mixed esters showed a small peak at carbon number 18.8 and a large peak at carbon number 19.7. The highly unsaturated C_{18} nonhydroxy acids (isanic and bolekic) run together at a carbon number of 19.7. Methyl ximenynate also gives carbon number 18.8 and when the mixed boleko esters were chromatographed with about 10% of methyl ximenynate, the small peak with carbon number 18.8 had completely merged with the large peak now obtained for methyl ximenynate. This indicates the presence of a small amount of ximenynic acid or a very similar acid in boleko oil. Since ximenynic and bolekic acids possess structural similarities, their co-occurrence is not surprising.

The hydrogenated esters showed a peak at 19.3 and another at 19.6. 12-ketostearate gives carbon number 19.3 and 12-hydroxystearate gives carbon number 19.6. The hydrogenated esters when rechromatographed with about 10% of 12-ketostearate and again with 10% of 12-hydroxystearate showed that the original peaks at carbon numbers 19.3 and

19.6, were identical with the peaks now given by the keto- and hydroxystearates respectively.

Carbon numbers. (5%. Apiezon L. column, at 200°C., argon flow-rate 83.3cm./min.)

Methyl esters	Pure esters	Mixed esters boleko acids	Hydrogenated esters boleko acids
Laurate	12.0	-	12.0
Myristate	14.0	14.0	14.0
palmitate	16.0	16.0	16.0
Oleate & linoleate	17.6	17.6	-
Stearate	18.0	18.0	18.0
Ximenynate	18.8	18.8	-
12-ketostearate	19.3	-	19.3
12-OH-stearate	19.6	19.7*	19.6
Di-OH-stearate	20.8	-	-

* = carbon number of isanic and bolekic acids, which run together.

The peak of carbon number 19.3 in the hydrogenated esters assigned to ketostearate deserves comment. This keto-acid is formed during hydrogenation of the mixed acids of boleko oil. In a recent report,[40] it has been shown that during hydrogenation of unsaturated hydroxy

acids, some keto acid is formed. This has been substantiated by certain experiments of Mr. Sealy; after hydrogenation of 3 different unsaturated hydroxy esters, each product contained some keto acid. In further experiments on boleko acids, Mr. Sealy concludes that the mixed acids do not contain any keto acid and the keto compound present in hydrogenated boleko acids is formed entirely during hydrogenation. The unsaturated hydroxy acids present in boleko acids are not satisfactorily eluted under the chromatographic conditions employed.

Quantitative examination.

Using a Pye-argon chromatograph fitted with an Integram, synthetic mixtures of fatty acids were first examined. Generally, mixtures of saturated, hydroxy and keto C_{18} esters gave incorrect results.[41] A number of standard mixtures were, therefore, examined and correction factors were determined for the hydroxy and keto stearates. Quantitative results on the standard mixtures were calculated by direct reading of the number of divisions moved by the pointer for each component in the mixture and the relative response of the beta-ray/ionisation detector to each functional group was determined. The detector used in these experiments responds to per cent. weights.

Standard mixtures.

Apiezon L. column (2½ % and 5 %), at 200 C., argon flow-rate 33.3 cm./min. Mixtures A, B, C, & D were analysed on a 5 % column, while mixture E was analysed on a 2½ % column.

Mixture	No. of determinations.	Saturated acids.	Actual %.wt.	Found %.wt.	Standard deviation.
A.	15	C_{16}	42.5	44.8	1.8
		C_{18}	57.5	55.2	1.7
B.	10	C_{18}	61.2	70.4	2.0
		C_{18}(12-keto)	38.8	29.6	2.0
C.	9	C_{18}	52.2	66.4	2.5
		C_{18}(12-OH)	47.8	33.6	2.5
D.	6	C_{18}	29.3	36.4	1.2
		C_{18}(12-keto)	38.1	40.1	1.7
		C_{18}(12-OH)	32.6	23.5	2.4
E.	8	C_{14}	1.0	0.9	2.0
		C_{16}	4.8	4.6	
		C_{18}	70.5	76.7	
		C_{18}(12-keto)	6.0	4.8	1.4
		C_{18}(12-OH)	17.7	12.9	1.6

Correction factors. The results for mixtures B, C, D and E, containing hydroxy and/or keto stearate are clearly unsatisfactory, probably because the response of the ionisation detector, though constant through a range of familiar saturated and unsaturated fatty acid esters, varies with hydroxy and keto esters. It is possible to calculate empirical correction factors for these two esters from the above results. These are not as consistant as could be wished but mixture E was specifically designed to simulate hydrogenated boleko acids and therefore the correction factors of 1.47 and 1.33 are used for hydroxy and keto stearates respectively.

Mixture	B.	C.	D.	E.
OH-stearate	-	1.42	1.73	1.47
Keto stearate	1.31	-	1.18	1.33.

Hydrogenated boleko esters. The G.L.C. shows 5 peaks corresponding to myristate, palmitate, ketostearate, stearate and hydroxy stearate and the average value for 6 determinations are given on page 98.

Mixed boleko esters. Four peaks were measured corresponding to (i) palmitate, (ii) linoleate, oleate and stearate taken together because they were not very well resolved, (iii) ximenynate and (iv) isanate and bolekate taken to-

-gether because they were not well resolved. The unsaturated hydroxy esters were not satisfactorily eluted and have therefore been neglected. The sum of the four measured peaks were adjusted to 72.4, the value obtained for the appropriate peaks in the chromatogram of the hydrogenated esters. The values are given on page 98 and results derived from these two sets of figures are given on page 79 (Column C).

Composition of boleko oil mixed esters before and after Hydrogenation.

(Pye-argon chromatograph fitted with an Integram; 3½ % Apiezon L. column, at 200 C., argon flow-rate 33.3 cm./ min.)

Hydrogenated boleko esters. 6 determinations)	Ester	Mixed boleko esters. (6 determinations)
0.4	Myristate	-
2.3	Palmitate	3.8
70.1	Linoleate	20.9
	Oleate	
	Stearate	
	Ximenynate	1.1
	Isanate	46.6
	Bolekate	
22.4	OH-stearate	-
4.8	Keto stearate	-
→	Undetected OH-acids	27.6*

* value obtained by difference.

Hydroxyl values.

Hydroxyl values were determined according to the method of Fritz and Schenk[42] on the methyl esters of hydrogenated M_1 fraction of boleko oil. All the solutions used were at the same strength as described by Fritz and Schenk, but the reactions were carried out on 1/10th. of the scale indicated and esters of the hydroxy material were used to avoid the necessity of determining the acid values. The details of the method are given on pp 37-39.

The method was applied to 12-hydroxystearic acid, threo 12,13,dihydroxy oleic acid and to the methyl esters of hydrogenated M_1 fraction of boleko oil, with the following results.

Material	% Hydroxyl
1. 12-hydroxystearic acid	4.8 (observed)
	5.7 (calculated)
2. threo 12,13,dihydroxyoleic acid	9.5 (observed)
	10.7 (calculated)
3. Methyl esters of hydrogenated M_1 fraction of boleko oil	5.4

The value of 5.4 % as hydroxyl value for the methyl esters of hydrogenated M_1 fraction of boleko oil could

indicate the presence of only the monohydroxy acid component; and this value compares well with the values (4.8% observed and 5.7% calculated) for 12-hydroxystearic acid.

Glycol values.

Glycol values of M_1 and M_2 fractions from the mixed acids of boleko oil were determined according to the method of Bharucha and Gunstone.[37]

Since the dihydroxy acid obtained from an epoxy acid is an alpha-glycol, it was thought desirable to determine the content of hydroxyl groups to find out if the M_1 and M_2 fractions contained epoxy acids. In addition, this would also differentiate between alpha-hydroxy acids and acids containing one or more hydroxyl groups not in adjacent positions.

Glycol values of dihydroxy stearic acid were first determined and then the values for M_1 and M_2 fractions from the mixed acids of boleko oil.

The details of the method are given on pp 39-40.

Sample	% Glycol
1. Dihydroxy stearic acid	98.0
2. Boleko mixed acids fractions	
M_1	7.9
M_1 hydrogenated	6.1
M_2	5.4
M_2 hydrogenated	5.5

The glycol values for the boleko oil fractions are

small and not constistant, compared with the high value for dihydroxy stearic acid. This could indicate the absence of dihydroxy acids in the boleko oil fractions. The value for the M_1 fraction is almost double the value for the M_2 fraction indicating a concentration of hydroxy acid(s) in the M_1 fraction.

References

1. Eckey, E. W., " Vegetable Fats and Oils", p. 394, Reinhold Publishing Corporation, 1954.
2. Hilditch, T. P., "The Chemical Constitution of Natural Fats", pp. 220, 536, 537, Chapman and Hall, 1956.
3. Gunstone, F. D., "An Introduction to the Chemistry of Fats and Fatty Acids", p. 21, Chapman and Hall, 1958.
4. Hebert, Bull. soc. chim., 1896, 15, 935; Compt. rend., 1896, 122, 155.
5. Steger, A., and J. van Loon, Fette u. Seifen, 1937, 44, 243; C.A., 32, 4366.
6. Boekenoogen, H.A., Fette u. Seifen, 1937, 44, 344; C.A., 32, 4367.
7. Castille, A., Annalen, 1939, 545, 104; C.A., 34, 2529.
8. Steger, A., and J. van Loon, Rec. trav. chim., 1940, 59, 1156; C.A., 35, 5095.
9. Steger, A., and J. van Loon, Rec. trav. chim., 1941, 60, 342; C.A., 35, 7745.
10. Steger, A., and J. van Loon, Rec. trav. chim., 1941, 60, 106; C.A., 36, 2526.
11. Castille, A., Bull. Acad. Roy. Med. Belg., 1941, 6, 152; C.A., 41, 3049.
12. Fauve, M., Peintures, Pigments, Vernis, 1948, 24, 147, 183, 209, 346, 281; C.A., 43, 3629.

13. Kaufmann, H.P., J.Baltes, and H.Herminghaus, Fette u. Seifen, 1951, 53, 537; C.A., 46, 6852.

14. Riley, J.P., J.Chem. Soc., 1951, 1346.

15. Armitage, J.B., C.L.Cook, N.Entwistle, E.R.H.Jones, and H.C.Whiting, J.Chem. Soc., 1952, 1998.

16. Black, H.K., and B.C.L.Weedon, J.Chem. Soc., 1953, 1785.

17. Seher, A., Fette u. Seifen, 1951, 54, 544; C.A., 48, 1952.

18. Seher, A., Annalen, 1954, 589, 222; C.A., 50, 1599.

19. Seher, A., Arch. Pharm., 1954, 287, 548; C.A., 52, 8946.

22. Meade, E.M., "Progress in the Chemistry of Fats and other Lipids", pp. 49-62, Pergamon Press, 1957.

23. Dupont, G., R.Dulou, and F.Pouliquen, Bull. soc. chim. France, 1957, 1496.

24. Vries, E. de, Oleagineux, 1957, 12, 427; C.A., 52, 7739.

25. Kneeland, J.A., D.Kyriocou, and R.H.Purdy, J.Am. Oil Chem. Soc., 1958, 35, 361.

27. Pouliquen, F., Oleagineux, 1959, 14, 381, 453, 541; C.A., 54, 5127.

28. Hatt, H.H., A.C.K.Triffett, and P.C.Wailes, Austr. J. Chem., 1959, 12, 190.

30. Hatt, H.H., A.C.K.Triffett, and P.C.Wailes, Austr. J. Chem., 1960, 13, 488.

31. Sorensen, N.A., Proc. Chem. Soc., 1961, 109.

32. Arnaud, A., Compt. rend., 1892, 114, 79; 1902, 134, 475.

33. Lighthelm, S.P., and Schwarz, H.M., J.Am. Chem. Soc., 1950, 72, 1868.

34. Lighthelm, S.P., Chem. and Ind., 1954, 247.

35. Gunstone, F.D., and M.A. McGee, Chem. and Ind., 1954, 1112.

36. Hatt, H.H., and Szumer, A.Z., Chem. and Ind., 1954, 963.

37. Bharucha, K.E., and Gunstone, F.D., J.Sci.Fd.Agric., 1955, 6, 373.

38. Pitt, G.A.J., and R.A. Morton, "Progress in the Chemistry of Fats and other Lipids", p. 242, Pergamon Press, 1957.

39. Woodford, F.P., and C.M. van Gent, J.Lipid Research, 1960, 1, 188.

40. Applewhite, T.H., M.J. Diamond, and L.A. Goldblatt, J.Am. Oil Chem. Soc., 1961, 38, 609.

41. Fontell, K., R.T. Holman, and G. Lambertsen, J.Lipid Research, 1960, 1, 391.

42. Frotz, J.S., and G.H. Schenk, Anal. Chem., 1959, 31, 1808.

Vernonia seed oils.

Vernonia Seed oils.

After the discovery of the first naturally occurring epoxy acid (72 %.) (cis 12-13, epoxyoctadec-9-enoic acid) by Gunstone[1] in the seed oil of Vernonia anthelmintica, other Vernonia seed oils have been examined. One of these, V. colorata[2] is reported to contain a high proportion of this acid, but the others (V. deppeana, V. fasciculata, and V. camporum)[3,4] contain little or none of it. It was therefore thought desirable to investigate other seed oils of this genus to see how general is the occurrence of this acid.

The same epoxyoleic acid occurs in the seed oils of Cephalocroton cordofanus (66 %)[5], Cephalocroton peuschelii (72.4 %)[4], Hibiscus species (okra, 3-4 %.; kenaf, 5 %.)[2,6], Clarkia elegans[7] and ten species of Malvaceae (negligible to 7 %.)[8].

Cis 9-10, epoxyoctadec-12-enoic acid, an isomer of epoxyoleic acid, is present in Chrysanthemum coronarium seed oil[9], and cis 9-10, epoxystearic acid has been identified among the mixed acids from uredospores of a wheat stem rust[10] and from Tragopogon porrifolius seed oil[11] and trans 9-10, epoxystearic acid has been isolated from orujo oil.[12]

15-16, Epoxyoctadec 9-12, dienoic acid is shown to

occur in the seed oil of Camelina sativa[13] and an undesignated epoxy acid (7.9 %) in Artemisia seed oil.[14]

Epoxy acids are thus shown to be constituents of a large number of seed oils belonging to different species[15-1] and it appears that such acids occur widely.

In the present investigation, seven Vernonia seed oils have been examined by reversed-phase partition chromatography, by gas-liquid chromatography and by determination of the content of epoxy acid by near infrared spectroscopy. The seeds analysed are:

1. V. anthelmintica
2. V. amygdalina
3. V. colorata
4. V. cinerea
5. V. biafrae
6. V. nigritana and
7. V. camporum.

V. anthelmintica seed oil has been the subject of several investigations (see Gunstone[1] and other references there and also references 5, 18, and 20) and the content of epoxy acid, as reported, varies from 64 to 80 %. of the mixed acids.

V. colorata seed oil was examined by Chisholm and Hopkins[2] and shown to contain a high proportion of epoxy-

oleic acid. It gave a strong signal at 66 c.p.s. in proton magnetic resonance spectra.

V. camporum seed oil[4] was examined by R.P.C. and the absence of epoxy acid was confirmed by the infrared spectrum of the mixed acids and by the epoxide value.[21]

There is no report on the remaining Vernonia seed oils under investigation.

The seeds for the present investigation were obtained from Poona, India (V. cinerea) and Sierre Leone (V. colorata, V. amygdalina, V. biafrae, and V. nigritana); V. anthelmintica and V. camporum seed oils were available from previous investigations.

The seed oils were extracted and the mixed acids (excluding nonsaponifiable material) were isolated as described on pages 29-30. The results are summarised on the next page.

Summary of results.

(a). Extraction of Vernonia seeds. 9 species.

Vernonia species	oil %	wt. of oil saponified(g)	mixed acids g.%	nonsap.mat. g.%
V. biafrae	1.6	1.74	30.4	64.9
V. nigritana	7.6	3.80	59.7	13.5
V. colorata	3.1	1.49	47.1	38.0
V. amygdalina	5.0	2.29	61.9	25.2
V. cinerea	3.8	2.52	27.3	69.2
V. anthelmintica*	26.0-27.0%	-	-	7.0
V. camporum*	8.4	-	-	2.5
V. deppeana*	20.0	-	-	-
V. fasciculata*	28.0	-	-	-

V. biafrae : The seeds with fibrous matter extracted; pale green oil slightly aromatic.

V. nigritana: Only seeds extracted; pale brown oil very slightly aromatic.

V. colorata: Seeds partly separated from fibrous material extracted; pale brown oil slightly aromatic.

V. amygdalina: Only seeds extracted; pale brown oil slightly aromatic.

V. cinerea: Only seeds extracted; pale green oil with aromatic smell.

* V. anthelmintica: data from Gunstone[1]

* V. camporum: data from Gunstone and Sykes.[4]

* V. deppeana: data from Earle et. al.[3]

* V. fasciculata: data from Earle et. al.[3]

(b). Near infrared measurements of epoxy acid.

The epoxide content of the mixed esters, measured as described on page 34, is as follows.

	% epoxide.
V. anthelmintica	68
V. amygdalina	50
V. colorata	14
V. cinerea	19
V. biafrae	nil
V. nigritana	5
V. camporum	nil

(c). Gas-liquid chromatography.

Carbon numbers of peaks observed in chromatograms of mixed esters. (details on page 118)

9-10, epoxystearate: 19.0, 19.3.

V. anthelmintica: 14.0, 17.6, 18.0, 18.7, 19.1, 19.3.

V. amygdalina: 14.0, 16.0, 17.7, 18.0, 18.9, 19.1, 19.4, 19.9, 20.3.

V. biafrae: 14.0, 15.8, 16.0, 17.6, 17.7, 18.0, 20.0

V. colorata: 14.0, 15.8, 16.0, 17.6, 17.7, 18.0, 18.8, 19.0, 19.3, 19.8, 20.5.

V. cinerea: 11.0, 13.0, 14.0, 16.0, 17.5, 17.7, 18.0, 18.8, 19.0, 19.8, 20.2.

V. nigritana: 12.0, 13.3, 14.0, 15.2, 16.0, 16.7, 17.0, 17.6, 18.0, 18.4, 18.8, 19.1, 19.3, 19.8, 20.2, 20.6.

V. camporum: 16.0, 17.6, 17.7, 18.0.

The underlined carbon numbers represent large peaks.

(d). R.P.C. results.

(on next page)

Component acids. (%. mol)

Acid	oxygenated	C_{14}	C_{16}	C'_{16}	C_{18}	C'_{18}	C''_{18}	C_{20}	C_{22}
V. anthelmintica	79	1	6	-	2	1	9	1	1
V. amygdalina	56	1	9	-	5	6	22	1	1
V. colorata	35	11	13	5	6	12	15	2	2
V. cinerea	26	9	25	-	8	4	22	3	3
V. biafrae	12	7	22	11	8	15	20	2	3
V. nigritana	7	3	19	-	8	19	42	1	1

Component acids. (%. wt)

V. anthelmintica	80	1	5	-	2	1	9	1	1
V. amygdalina	58	1	8	-	5	6	20	1	1
V. colorata	38	9	11	5	6	12	15	2	2
V. cinerea	28	8	23	-	8	4	22	3	4
V. biafrae	13	6	21	10	8	15	21	2	4
V. nigritana	8	2	18	-	8	19	43	1	1
V. camporum *	-	-	14	-	9	22	55	-	-

* data from Gunstone and Sykes.[4]

Discussion.

1. Since the amount of mixed acids available in the present investigation of the six varieties of Vernonia oils was very small, it was not possible to measure the saponification equivalents and the iodine values in all cases. However, iodine values were determined for the mixed acids of V. amygdalina (93.2) and V. nigritana (93.0) and compared with the calculated iodine values (91.7 and 100.7 respectively). The calculated iodine value (93.2) for V. anthelmintica mixed acids is compared with the value (111.1) reported by Gunstone[1] for mixed acids containing (7.9 %.) unsaponifiable matter.

Since the saponification equivalents were not determined, the results for ozonised mixed acids were calculated by making the value for the C_{18} acid observed in the chromatogram for these acids agree with the value for the same acid (acid group) observed in the chromatogram for the mixed acids, assuming the absence of C_{20} mono and C_{22} di-ethenoid acids.

In these calculations, it has also been assumed that C_{12} and lower acids are absent.

2. The percentage of epoxy acids measured by near infra-red spectra for each oil is lower than the values obtained for oxygenated acids by R.P.C. This fact could be explained

in two ways.

(a) As the percent. epoxide in a sample increases, the background absorption also increases in the untreated sample; and as a result, the general background absorptivity (a_u) of the untreated sample is higher than that for the treated sample,[17] and the absorptivity ($a_t - a_u$) due to chlorohydrin, therefore, becomes lower.

(b) The G.L.C. of the esters of most of the samples investigated show the presence of small amounts of slower moving acid(s). Previous studies[5,24] have shown that epoxy acids are generally accompanied by small amounts of dihydroxy acids, which may have been formed from the epoxy acid during storage of the seeds or during investigation of the seed oil. This fact has not been taken into consideration in the calculations, since further investigation into the nature of these slower moving acids was precluded due to want of enough material. The oxygenated acids were, therefore, calculated as mainly epoxyoleic acids.

3. In the mixed acids chromatograms (R.P.C.), the C_{14} group of acids are resolved into two peaks. The acids eluted in these peaks were analysed by G.L.C. For example, esters of acids isolated from fractions 63-77 and 79-91, from the mixed acids chromatogram of V. colorata, showed the presence of myristic acid with traces of hexadecenoic

and linoleic acids in the first fraction and hexadecenoic and linoleic acids along with a trace of myristic acid in the second fraction. Carbon numbers of the peaks observed in the chromatograms of the two fractions are given below.

Fraction		carbon numbers.
1.	65-77	: 12.0, 14.0, 14.8, 15.7, 16.0, 17.0, 17.4, 17.8, 18.0, 19.0.
2.	79-91	: 11.6, 12.6, 13.4, 13.8, 14.0, 15.0, 15.7, 16.0, 16.5, 17.0, 17.4, 17.8, 18.8, 19.0.

Carbon numbers underlined indicate expected and large peaks; the remaining carbon numbers represent minor influxes in the chromatograms.

The observed values for the two peaks eluted in the mixed acids chromatograms (R.P.C.) are compared with the corresponding calculated values for myristic, hexadecenoic and linoleic acids.

Separation of myristic and linoleic acids by R.P.C.

	Two peaks eluted with 62 % acetone. (%.mol)		(C_{14}	C'_{16} %.mol	C''_{18})
V. anthelmintica	2	9	1	-	9
V. amygdalina	3	18	1	-	21
V. colorata	12	21	11	5	15
V. cinerea	8	22	9	-	22
V. biafrae	8	32	7	11	20
V. nigritana	---46---		5	-	42

In the mixed acids chromatogram (R.P.C.), it may be possible to separate myristic and linoleic acids quantitatively with suitable concentrations of the eluting solvent.

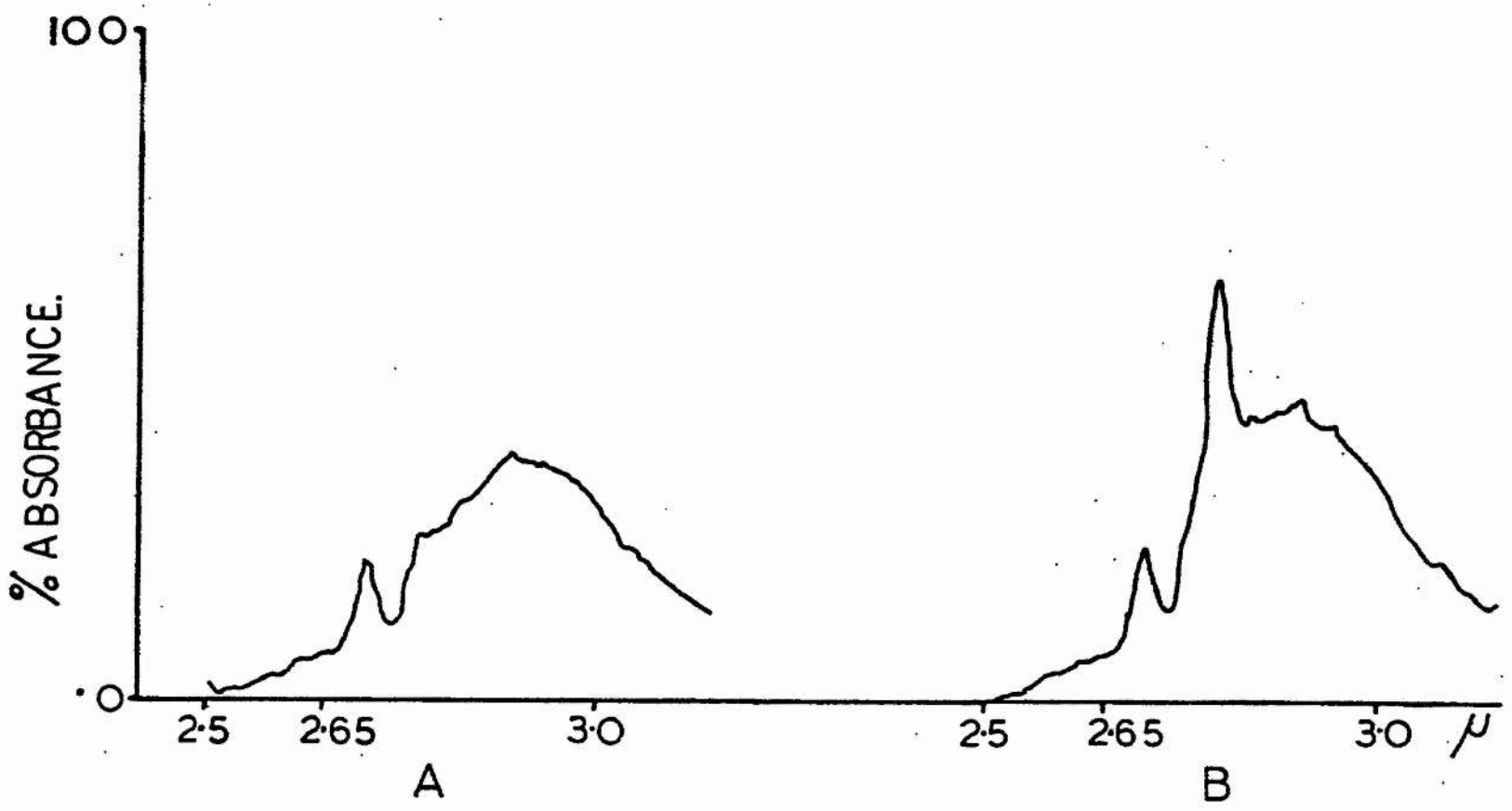
Detailed experimental results.

(a). Examination by gas-liquid chromatography.

(2½ %. Apiezon L. column, at 200 C., argon flow-rate 53.3 cm./ min., standard: methyl stearate.)

The methyl esters of the mixed acids of Vernonia oils were examined by G.L.C. and the carbon numbers[22] were calculated for the various peaks eluted in each chromatogram (see pages 111-112). These carbon numbers were compared with the carbon numbers for pure methyl 9:10 epoxystearate.

Excepting the esters of V. camporum and V. biafrae, all others show peaks for epoxy acid(s). V. colorata and V. nigritana esters also show minor peaks for slower moving acid(s) with carbon numbers at 20.5 and 20.6 respectively. Dihydroxy stearate has a carbon number of 20.8. Therefore, these slower moving acids could be dihydroxy acids. Bharucha and Gunstone[5] and Tulloch[24] have shown that epoxy acids are generally accompanied by small amounts of dihydroxy acids, which may have been formed from the epoxy acid during storage of the seeds or during investigation of the seed oil. The nature of these acid(s) could not be studied due to lack of material.



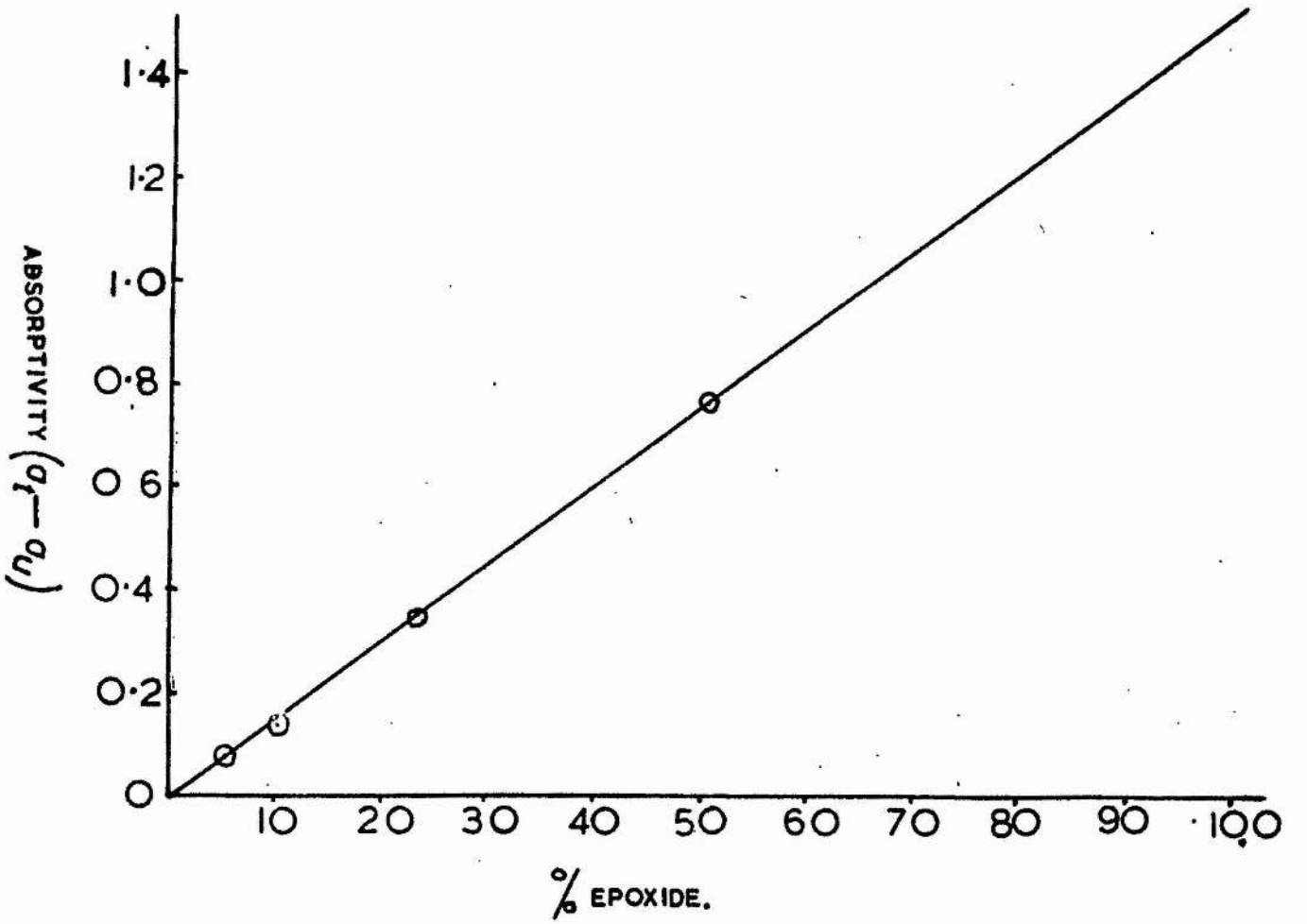
INFRARED SPECTRA OF V. ANTHELMINTICA BEFORE (A) AND AFTER (B) TREATMENT WITH HCl

(b). Epoxy values by near infrared spectroscopy.[17]

The near infrared spectra of oil samples as 3%, 1%, and 0.3% solutions in carbon tetrachloride were obtained on Grubb Parsons G. S. 2A. recording Spectrophotometer with glass cells of 1 cm. path length (KBr windows). The settings used throughout were scanning speed: 6"/u; gain 14/82; time constant 4.

The method (see pp 34-36) was first applied to pure olive oil, before and after treatment with hydrochloric acid at the same concentration, then to different solutions of 9:10 epoxystearic acid ester in olive oil (5%, 10%, 25% and 50%.). Absorptivity ($a_t - a_u$) due to chlorohydrin was calculated in each case, from the near infrared spectra for each sample before and after treatment with hydrochloric acid, both at the same concentration.

Material	Concn.	I.	T.	At.	Au.	At-Au.	at.	au.	at-au.
Olive oil									
untreated	0.3%	9.6	8.85	-	0.0354	-	-	-	-
Treated	0.3%	9.25	8.40	0.0418	"	0.0064	0.0139	0.0118	0.0021



CALIBRATION CURVE FOR INFRARED

DETERMINATION OF EPOXIDES.

Absorptivity of different solutions of 9:10 epoxystearate.

Material	Concentration	Absorptivity (at-au)
Olive oil	0.3 %	0.0021
5%. epoxystearate	0.3 %	0.0835
10%. epoxystearate	3.0%	0.1452
25%. epoxystearate	1.0 %	0.3870
50%. epoxystearate	0.3 %	0.7645
100%. epoxystearate	0.3 %	1.2650

A calibration curve was drawn by plotting absorptivity (at-au) against percent. of epoxystearic acid, which is a straight line. The observed value for 100% epoxystearic acid is low (mean of 16 determinations) and does not fall on the calibration line. This low value is not unexpected. Morris and Holman[17] observe that as the epoxide content in a sample increases, the background absorption also increases in the untreated sample; thus the general background absorptivity of the untreated oil sample is higher than that for the treated sample, and the absorptivity due to chlorohydrin, therefore, becomes lower.

Epoxy acids in Vernonia oils.

Absorptivity (at-au) was calculated in each case from the near infrared spectra of the sample before and

after treatment with hydrochloric acid and percentage of epoxy acid in the sample was determined by reading off from the above calibration curve (see figure opposite page 120). The observed epoxide values are compared with percentages of total oxygenated acids determined by R.P.C.

Sample	Concn. %	absorptivity (at-au)	% epoxide	% oxygenated acid
V. anthelmintica	0.5	1.0510 (mean of 1.0567, 1.005)	68	80
V. amygdalina	0.5	0.7564	50	58
V. cinerea	1.0	0.2130	19	28
V. colorata	1.0	0.1748	14	38
V. nigritana	1.0	0.0710	5	8
V. biafrae	1.0	0.0019	nil	13
V. camporum	3.0	0.0014	nil	nil

(c). Vernonia anthelmintica seed oil

1. Examination by gas-liquid chromatography.

The G.L.C. of the mixed esters of V. anthelmintica shows

(a) a very large peak at carbon number 19.1 (epoxy-oleic) and minor influxes at carbon numbers 18.7 and 19.4 (epoxylinoleic and epoxystearic respectively) and

(b) peaks at C'_{18}, C''_{18}, C_{18}, C_{16}, and and very small influxes at C_{14}, C_{20} and C_{22}.

2. Near infrared measurement of epoxy acids.

1. at-au = 1.0567
2. at-au = 1.0050

mean = 1.031

From the calibration curve, 1.031 = 68 %. epoxide.

3. Examination by R.P.C.

Summary (%. mol)

Acid group	10:0	12:0	14:0	16:0	18:0	20:0	22:0
Eluting solvent% (acetone)	43	53	62	67	73	78	83
Acetylated, hydrogenated acids.	66.9	13.9	0.6	5.5	11.0	1.2	0.9
Mixed acids.	0.8	77.1	10.6	7.6	2.3	0.8	0.8
Ozonised acids.	-	16.3	1.9	5.7	2.3	0.5	0.3

These figures give the results on page 113. It has been assumed that lauric and lower acids are absent. The

oxygenated acids are eluted in two portions after hydogenation (66.9% with 45% acetone, and 13.9% with 53% acetone).

Assuming the oxygenated acids to be entirely epoxyoleic acid, the saponification equivalent (291.7) and the iodine value (95.2) were calculated and compared with the values recorded by Gunstone[1] for mixed acids (excluding nonsaponifiable matter, S.E. 289.5) and for mixed acids containing 7.9 % unsaponifiable matter (iodine value, 111.1).

The results obtained in the present investigation of V. anthelmintica seed oil are compared with those reported in previous studies.

Component acids(% wt.) of V. anthelmintica seed oil.

Reference acid	Present work	1	23	5	18	20
Myristic	1	0.5	7.5	-	-	-
Palmitic	5	3.5	7.3	4	-	-
Stearic	2	1.5	6.0	2	-	-
Oleic	1	6.0	5.8	4	-	-
Linoleic	9	16.5	9.8	16	-	-
Arachidic	1	-	Tr	1	-	-
Behenic	1	-	-	-	-	-
Oxygenated (epoxyoleic)	80	72.0	63.6	73.0	73.0	79-80

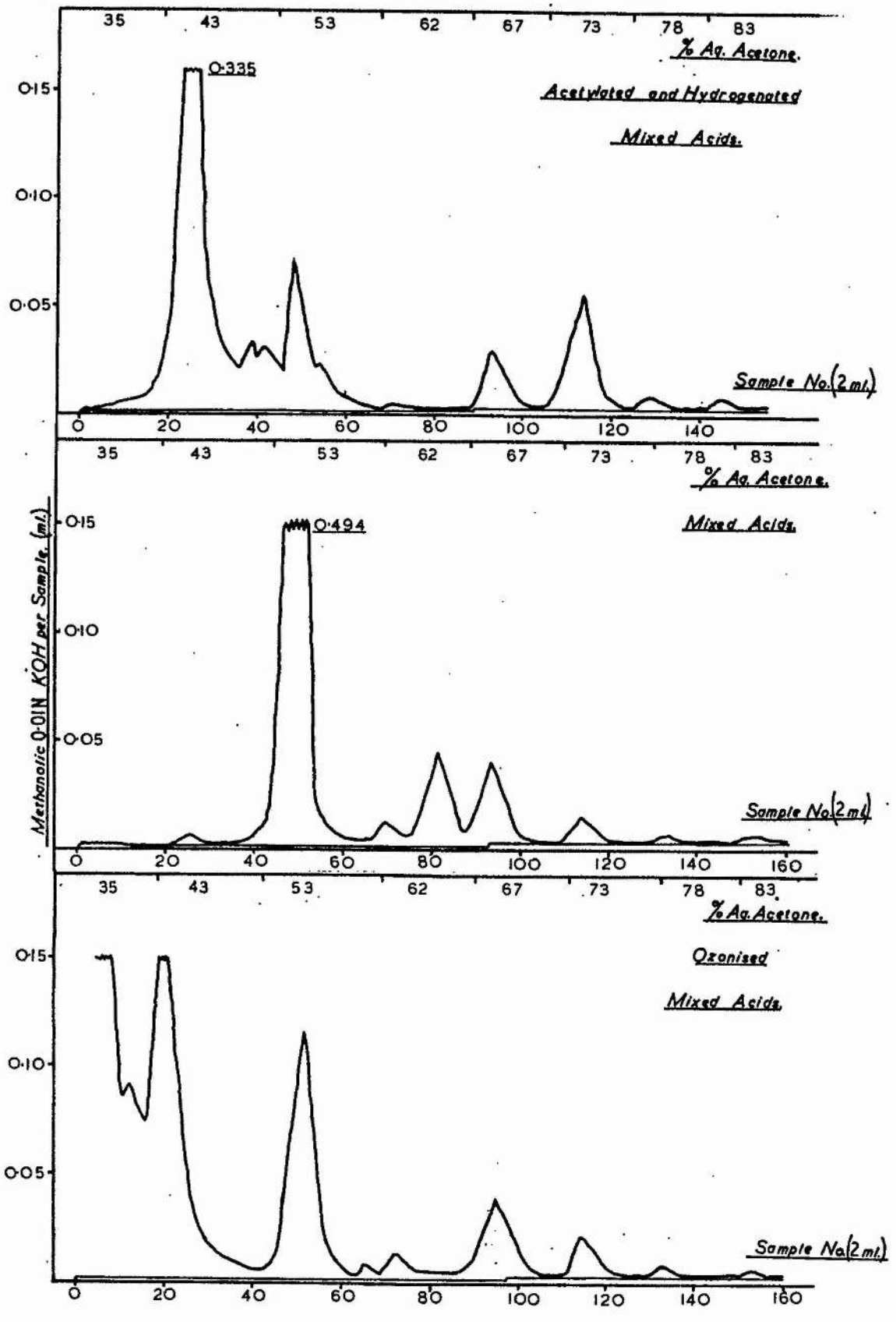


FIG.6 VERNONIA ANTHELMINTICA ACIDS.

Detailed R.P.C. results.

Acetylated, hydrogenated acids (22.6mg.), Alkali 1.797 x 10^{-2}

Acid group	Fr.No.	Tot.Alk.	Blank	Alk.Corr.	Eq x 10^{-5}	Mol%
10:0	16-35	1.868	0.057	1.851	3.290	59.1
	36-45	0.263	0.020	0.243	0.437	7.8
12:0	46-52	0.334	0.014	0.320	0.575	10.3
	53-66	0.141	0.028	0.113	0.203	3.6
14:0	67-75	0.035	0.018	0.017	0.031	0.6
16:0	87-103	0.218	0.049	0.169	0.304	5.5
18:0	104-123	0.400	0.060	0.340	0.611	11.0
20:0	124-135	0.073	0.036	0.037	0.066	1.2
22:0	141-150	0.059	0.030	0.029	0.052	0.9

Mixed acids (20.1mg.), Alkali 1.797 x 10^{-2} N.

Acid group	Fr.No.	Tot.Alk.	Blank	Alk.Corr.	Eq x 10^{-5}	Mol%
10:0	21-30	0.046	0.020	0.026	0.047	0.8
12:0	55-65	2.564	0.066	2.498	4.489	76.9
14:0	66-74	0.083	0.018	0.065	0.117	2.0
	75-87	0.306	0.026	0.280	0.503	8.6
16:0	88-107	0.307	0.059	0.248	0.446	7.6
18:0	108-121	0.117	0.042	0.075	0.135	2.3
20:0	129-140	0.063	0.036	0.027	0.048	0.8
22:0	147-157	0.060	0.033	0.027	0.048	0.8

Ozonised acids (55.4mg.), Alkali 1.797×10^{-2} N.

Acid	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq $\times 10^{-5}$	Mol%
12:0	42-63	0.792	0.044	0.748	1.344	15.8
	64-69	0.036	0.012	0.024	0.043	0.5
14:0	70-84	0.120	0.030	0.090	0.162	1.9
16:0	85-105	0.325	0.057	0.268	0.482	5.7
18:0	110-125	0.157	0.048	0.109	0.196	2.3
20:0	128-137	0.052	0.030	0.022	0.040	0.5
22:0	149-156	0.038	0.024	0.014	0.025	0.3

****

(d). Vernonia amygdalina seed oil.

1. Examination by gas-liquid chromatography.

The G.L.C. of the mixed esters of V. amygdalina shows

(a) a major peak at 19.1 (epoxyoleic) and minor influxes at carbon numbers 18.9 and 19.4 (epoxylinoleic and epoxystearic respectively), and

(b) peaks at carbon numbers 16.0, 17.7, 18.0, 19.9 & a slower moving acid at 20.5.

2. Near infrared measurement of epoxy acids.

(see table on page 121)

3. Examination by R.P.C.

Summary (% mol)

Acid group	10:0	12:0	14:0	16:0	18:0	20:0	22:0
Eluting solvent% (acetone)	48	53	6%	67	73	78	83
Acetylated, hydrogenated acids.	47.3	4.1	2.2	9.0	33.5	1.9	2.0
Mixed acids.	5.6	55.0	21.1	14.2	4.5	0.7	0.9
Ozonised acids.	-	4.4	0.8	9.9	4.5	1.3	0.4

These figures give the results on page 113. It has been assumed, in these calculations, that lauric and lower acids are absent. The oxygenated acids are eluted in two

portions after hydrogenation (47.5% with 45% acetone and 4.1% with 55% acetone.).

	Calculated	Observed
Saponification equivalent	287.7	-
Iodine value	91.7	93.2

Detailed R.P.C. results.

Acetylated, hydrogenated acids (25.8mg.), Alkali 1.797×10^{-1}

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq $\times 10^{-5}$	Mol%
10:0	22-35	1.171	0.055	1.116	2.005	39.2
	36-48	0.282	0.052	0.230	0.413	8.1
12:0	49-57	0.148	0.036	0.112	0.211	4.1
14:0	73-81	0.106	0.045	0.061	0.110	2.2
16:0	92:106	0.330	0.075	0.255	0.458	9.0
18:0	107-131	1.098	0.143	0.955	1.716	33.5
20:0	132-140	0.109	0.054	0.055	0.099	1.9
22:0	144-156	0.135	0.078	0.057	0.102	2.0

Mixed acids (21.2mg.), Alkali 1.797 x 10^{-2} N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol %
10:0	18-30	0.156	0.039	0.117	0.210	3.6
12:0	51-60	1.784	0.090	1.694	3.044	52.9
	61-67	0.087	0.021	0.066	0.119	2.1
14:0	68-72	0.117	0.020	0.097	0.174	3.0
	73-84	0.629	0.048	0.581	1.044	18.1
16:0	85-99	0.517	0.060	0.457	0.821	14.2
18:0	100-119	0.251	0.105	0.146	0.262	4.5
20:0	120-126	0.057	0.035	0.022	0.040	0.7
22:0	134-142	0.075	0.045	0.030	0.054	0.9

Ozonised acids (46.5mg.), Alkali 1.797 x 10^{-2} N.

Acid	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol %
12:0	48-55	0.108	0.032	0.076	0.136	1.1
	56-72	0.290	0.068	0.222	0.400	3.3
14:0	73-81	0.102	0.045	0.057	0.102	0.8
16:0	90-109	0.768	0.100	0.668	1.200	9.9
18:0	110-128	0.416	0.114	0.302	0.543	4.5
20:0	129-143	0.177	0.090	0.087	0.156	1.3
22:0	144-157	0.175	0.084	0.091	0.164	1.4

(e). Vernonia colorata seed oil.

1. Examination by gas-liquid chromatography.

The G.L.C. of the mixed esters of V. colorata shows

(a) a peak at carbon number 19.0 (19.1) with minor influxes at carbon numbers 18.8 and 19.3 and also a slower moving acid at carbon number 20.5, and

(b) peaks at 14.0, 15.8, 16.0, 17.6, 17.7, and 18.0.

2. Near infrared measurement of epoxy acids.

(see page 121)

3. Examination by R.P.C.

Summary (% mol)

Acid group	10:0	12:0	14:0	16:0	18:0	20:0	22:0
Eluting solvent% (acetone)	43	53	62	67	73	78	83
Acetylated, hydrogenated acids.	21.8	15.9	10.0	16.9	32.4	1.6	1.4
Mixed acids.	20.0	14.2	32.9	23.8	5.5	1.7	1.9
Ozonised acids.	--	2.8	12.1	12.3	5.5	1.8	1.6

These figures give the results on page 113. In the calculations, it has been assumed that lauric and lower acids are absent. The oxygenated acids are eluted in two portions after hydrogenation (21.8% with 43% acetone and 15.9% with 53% acetone). Saponification equivalent(278.3) and iodine value(74.7) are calculated.

Detailed R.P.C. results.

Acetylated, hydrogenated acids. (20.5mg.), Alkali 1.862 x 10^{-2}

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol %
10:0	2-10	0.074	0.018	0.056	0.104	2.2
	17-34	0.538	0.036	0.502	0.934	19.6
12:0	35-53	0.446	0.038	0.408	0.760	15.9
14:0	54-73	0.313	0.056	0.257	0.478	10.0
16:0	74-95	0.498	0.066	0.432	0.804	16.9
18:0	100-123	0.925	0.095	0.828	1.542	32.4
20:0	124-134	0.085	0.044	0.041	0.076	1.6
22:0	141-151	0.080	0.044	0.036	0.067	1.4

Mixed acids. (19.9mg.), Alkali 1.797 x 10^{-2} N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol %
10:0	1-13	0.252	0.013	0.239	0.429	6.3
	14-20	0.092	0.007	0.085	0.153	2.2
	21-43	0.458	0.023	0.435	0.781	11.5
12:0	44-60	0.555	0.017	0.538	0.966	14.2
14:0	64-79	0.464	0.029	0.435	0.781	11.5
	80-99	0.852	0.040	0.812	1.460	21.4
16:0	100-119	0.942	0.040	0.902	1.621	23.8
18:0	120-135	0.254	0.043	0.211	0.379	5.5
20:0	139-151	0.105	0.039	0.066	0.118	1.7
22:0	156-168	0.112	0.039	0.073	0.131	1.9

Ozonised acids. (32.4mg.), Alkali 1.797×10^{-2} N.

Acid	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq $\times 10^{-5}$	Mol %
12:0	45-59	0.231	0.060	0.171	0.307	2.8
14:0	60-90	0.873	0.149	0.724	1.301	12.1
16:0	91-117	0.871	0.135	0.736	1.323	12.3
18:0	118-140	0.470	0.134	0.336	0.604	5.5
20:0	141-157	0.209	0.104	0.105	0.192	1.8
22:0	158-172	0.185	0.090	0.095	0.171	1.6

****

(I). Vernonia cinerea seed oil.

1. Examination by gas-liquid chromatography.

The G.L.C. of the mixed esters of V. cinerea shows (a) small influxes at carbon numbers 18.8 and 19.1 and (b) peaks at carbon numbers 14.0, 16.0, 17.5, 17.7, 18.0, 19.8, 20.2 and also at 11.0, and 13.0,

2. Near infrared measurement of epoxy acids.

(see page 121)

3. Examination by R.P.C.

Summary (% mol)

Acid group	10:0	12:0	14:0	16:0	18:0	20:0	22:0
Eluting solvent% (acetone)	45	55	62	67	73	78	83
Acetylated, hydrogenated acids.	14.7	11.4	8.8	25.1	33.0	5.8	3.2
Mixed acids.	4.6	24.0	30.0	28.4	8.5	2.6	1.9
Ozonised acids.	-	6.5	9.4	25.7	8.5	3.0	2.0

These figures give the results on page 113. In the calculations, it has been assumed that lauric and lower acids are absent. The oxygenated acids are eluted in two portions after hydrogenation (14.7% with 45% acetone and 11.4% with 55% acetone). The saponification equivalent (276.9) and the iodine value (67.8) are calculated.

Detailed R.P.C. results.

Acetylated hydrogenated acids (19.8mg.), Alkali 1.797 x 10^{-2} N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol%
10:0	16-34	0.407	0.036	0.371	0.667	14.7
12:0	35-60	0.540	0.052	0.288	0.518	11.4
14:0	61-78	0.259	0.036	0.223	0.401	8.8
16:0	79-106	0.717	0.083	0.634	1.139	25.1
18:0	107-129	0.906	0.069	0.837	1.504	33.0
20:0	130-145	0.143	0.048	0.095	0.171	3.8
22:0	146-158	0.119	0.039	0.080	0.144	3.2

Mixed acids. (21.7mg.), Alkali 1.797 x 10^{-2} N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol%
10:0	13-24	0.210	0.044	0.166	0.298	4.6
12:0	35-53	0.942	0.076	0.866	1.558	24.0
14:0	54-67	0.369	0.070	0.299	0.537	8.3
	68-80	0.849	0.065	0.784	1.409	21.7
16:0	81-97	1.107	0.085	1.022	1.836	28.4
18:0	98-110	0.386	0.078	0.308	0.553	8.5
20:0	111-120	0.155	0.060	0.095	0.171	2.6
22:0	121-130	0.127	0.060	0.067	0.120	1.9

Ozonised acids. (40.2mg.), Alkali 1.797 x 10^{-2} N.

Acid	Fr.No.	Tot.Alk.	Blank	Alk.Corr.	Eq x 10^{-5}	Mol%
12:0	35-53	0.381	0.076	0.305	0.548	6.5
14:0	54-64	0.356	0.055	0.301	0.541	6.5
	65-74	0.186	0.050	0.136	0.244	2.9
16:0	75-93	1.296	0.095	1.201	2.158	25.7
18:0	94-109	0.495	0.096	0.397	0.713	8.5
20:0	110-122	0.220	0.078	0.142	0.255	3.0
22:0	123-155	0.174	0.078	0.096	0.173	2.0

****

(g). Vernonia biafrae seed oil.

1. Examination by gas-liquid chromatography.

The G.L.C. of the mixed esters of V. biafrae shows

(a) peaks at carbon numbers 14.0, 15.8, 16.0, 17.6, 17.7, 18.0, and 20.0, and

(b) the absence of epoxy acid(s).

2. Near infrared measurement of epoxy acids.

(see page 121)

3. Examination by R.P.C.

Summary (% mol)

Acid group	10:0	12:0	14:0	16:0	18:0	20:0	22:0
Eluting solvent% (acetone)	43	53	62	67	73	78	83
Acetylated, hydrogenated acids.	2.3	2.4	6.9	34.8	48.0	2.4	3.3
Mixed acids.	8.0	3.2	40.7	35.1	8.4	2.1	2.5
Ozonised acids.	-	1.5	5.4	21.6	8.4	1.6	2.6

These figures give the results on page 113. In these calculations, it has been assumed that lauric and lower acids are absent. The oxygenated acids elute in two portion after hydrogenation (2.3% with 43% acetone and 2.4% with 53% acetone). The saponification equivalent (273.3) and iodine value (72.5) are calculated from these results

Detailed R.P.C. results.

Acetylated, hydrogenated acids. (23.5mg.), Alkali 1.797 x 10^{-2}

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol%
10:0	15-30	0.109	0.030	0.079	0.142	2.3
12:0	45-57	0.108	0.026	0.082	0.147	2.4
14:0	67-77	0.182	0.033	0.149	0.268	4.3
	78-85	0.115	0.024	0.091	0.164	2.6
16:0	86-115	1.296	0.090	1.206	2.167	34.8
18:0	116-142	1.770	0.108	1.662	2.987	48.0
20:0	143-155	0.154	0.052	0.082	0.147	2.4
22:0	161-175	0.173	0.060	0.113	0.203	3.3

Mixed acids. (21.7mg.), Alkali 1.797 x 10^{-2}N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol%
10:0	16-41	0.311	0.049	0.262	0.471	8.0
12:0	42-62	0.148	0.042	0.106	0.190	3.2
14:0	63-74	0.223	0.032	0.191	0.343	5.8
	75-77	0.077	0.009	0.068	0.122	2.1
	78-97	1.139	0.060	1.079	1.939	32.8
16:0	98-119	1.223	0.066	1.157	2.079	35.1
18:0	120-140	0.357	0.080	0.277	0.498	8.4
20:0	141-158	0.138	0.072	0.066	0.119	2.1
22:0	159-170	0.132	0.048	0.084	0.151	2.5

Ozonised acids. (30.1mg.), Alkali 1.797 x 10^{-2} N.

Acid	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol %
12:0	44-58	0.101	0.030	0.071	0.128	1.5
14:0	65-75	0.171	0.029	0.142	0.255	3.0
	76-87	0.150	0.036	0.114	0.205	2.4
16:0	88-112	1.085	0.075	1.010	1.815	21.6
18:0	113-129	0.461	0.068	0.393	0.706	8.4
20:0	130-145	0.137	0.064	0.075	0.151	1.6
22:0	146-165	0.202	0.080	0.122	0.219	2.6

(h). Vernonia nigritana seed oil.

1. Examination by gas-liquid chromatography.

(a) The G.L.C. of the mixed esters of V. nigritana shows

(a) minor influxes at carbon numbers 18.8, 19.1, 19.3 and slower moving acids with carbon numbers at 19.8, 20.2 and 20.6, and

(b) peaks at carbon numbers 16.0, 17.6, 18.0 and smaller influxes at 12.0, 13.3, 14.0, 15.2, 16.7, 17.0, and 18.4.

2. Near infrared measurement of epoxy acids.

(see page 121)

3. Examination by R.P.C.

Summary (% mol)

Acid group No.	10:0	12:0	14:0	16:0	18:0	20:0	22:0
Eluting solvent% (acetone)	43	55	62	67	73	78	83
Acetylated, hydrogenated acids.	7.2	3.1	3.0	18.4	67.2	0.7	0.4
Mixed acids.	4.2	1.9	46.2	39.1	7.4	1.1	0.1
Ozonised acids.	-	5.3	2.0	18.1	7.4	1.2	0.9

These figures give the results on page 113. In these calculations, it has been assumed that lauric and lower acids are absent. The oxygenated acids are eluted in two

portions after hydrogenation (7.2% with 43% acetone and 3
with 53% acetone).

	calculated	observed
Saponification equivalent	277.0	-
Iodine value	100.7	93.0

Detailed R.P.C. results.

Acetylated hydrogenated acids (20.1mg.), Alkali 1.797 x 10^{-2}

Acid group	Fr.No.	Tot.Alk.	Blank	Alk.Corr.	Eq x 10^{-5}	Mol%
10:0	12-31	0.393	0.132	0.261	0.468	4.9
	32-49	0.246	0.126	0.120	0.216	2.3
12:0	50-65	0.278	0.112	0.166	0.298	3.1
14:0	66-86	0.316	0.156	0.160	0.288	3.0
16:0	87-113	1.201	0.216	0.985	1.762	18.4
18:0	114-148	3.875	0.305	3.570	6.420	67.2
20:0	149-164	0.180	0.144	0.036	0.065	0.7
22:0	165-179	0.158	0.135	0.023	0.041	0.4

Mixed acids. (26.3mg.), Alkali 1.797×10^{-2} N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol%
10:0	17-40	0.426	0.104	0.322	0.579	4.2
12:0	41-60	0.246	0.100	0.146	0.262	1.9
14:0	77-108	3.757	0.192	3.565	6.406	46.2
16:0	109-135	3.185	0.162	3.023	5.432	39.2
18:0	136-156	0.711	0.137	0.574	1.031	7.4
20:0	157-179	0.244	0.161	0.083	0.149	1.0
22:0	180-189	0.078	0.070	0.008	0.014	0.1

Ozonised acids. (53.2mg.), Alkali 1.797×10^{-2} N.

Acid	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol%
12:0	45-71	0.586	0.135	0.451	0.810	3.2
14:0	72-92	0.409	0.126	0.283	0.509	2.0
16:0	93:123	2.706	0.186	2.520	4.528	18.4
18:0	124-150	1.210	0.182	1.028	1.847	7.4
20:0	151-168	0.292	0.126	0.166	0.298	1.2
22:0	169-187	0.261	0.135	0.126	0.230	0.9

****

(1). Vernonia camporum seed oil.

1. Examination by gas-liquid chromatography.

The G.L.C. of the mixed esters of V. camporum shows

(a) no peaks for epoxy acid(s), and

(b) peaks at carbon numbers 16.0, 17.6, 17.7, and 18.

2. Near infrared measurement of epoxy acids.

(see page 121)

3. Examination by R.P.C.

(data from Gunstone and Sykes.[4])

****

References

1. Gunstone, F.D., J. Chem. Soc., 1954, 1611.

2. Chisholm, M.L., and Hopkins, C.Y., Can. J. Chem., 1957, 35, 358.

3. Earle, F.R., C.A. Glass, G.C. Geisinger, and Wolff, I.A., J. Am. Oil Chem. Soc., 1960, 37, 440.

4. Gunstone, F.D., and Sykes, P.J., J. Sci. Fd. Agric., 1961, 12, 115.

5. Bharucha, K.E., and Gunstone, F.D., J. Sci. Fd. Agric., 1956, 7, 606.

6. Hopkins, C.Y., and Chisholm, M.L., J. Am. Oil Chem. Soc., 1959, 36, 95.

7. Smith, C.R. Jr., Bagby, M.O., Lohmar, R.L., Glass, C.A., and Wolff, I.A., J. Org. Chem., 1960, 25, 218.

8. Hopkins, C.Y., and Chisholm, M.L., J. Am. Oil Chem. Soc., 1960, 37, 682.

9. Smith, C.R. Jr., K.F. Koch, and Wolff, I.A., Chem. and Ind., 1959, 259.

10. Tulloch, A.P., B.M. Craig, and Ledingham, C.A., Can. J. Microbiol., 1959, 5, 485.

11. Chisholm, M.L., and Hopkins, C.Y., Chem. and Ind., 1959, 1154.

12. Vioque, E., L.J. Morris, and Holman, R.T., J. Am. Oil Chem. Soc., 1961, 38, 489.

13. Gunstone, F.D., and Morris, L.J., J. Chem. Soc., 1959, 2127.

14. Vioque, E., and Holman, R.T., J. Am. Oil Chem. Soc., 1962, 39, 63

15. Earle, F.R., Melvin, E.H., Mason, L.H., C.A. van Etten, I.A. Wolff, and Jones, Q., J. Am. Oil Chem. Soc., 1959, 36, 304.

16. Smith, C.R. Jr., M.C. Burnett, T.L. Wilson, R.L. Lohmar, and Wolff, I.A., J. Am. Oil Chem. Soc., 1960, 37, 320.

17. Morris, L.J., and Holman, R.T., J. Lipid Research, 1961, 2, 77.

18. Morris, L.J., Holman, R.T., and Fontell, K., J. Lipid Research, 1961, 2, 68.

19. Morris, L.J., Holman, R.T., and Fontell, K., J. Am. Oil Chem. Soc., 1960, 37, 323.

20. Morris, L.J., H. Heyes, and Holman, R.T., J. Am. Oil Chem. Soc., 1961, 38, 316.

21. King, G., Nature, 1949, 164, 706; J. Chem. Soc., 1951, 1980.

22. Woodford, F.P., and van Gent, C.M., J. Lipid Research, 1960, 1, 188.

23. Vidyarthi, N.L., Patna Univ. J., 1945, 1, 51.

24. Tulloch, A.P., Can. J. Chem., 1960, 38, 204.

Amburana Seed Oil.

Torresea cearensis.

Torresea cearensis Fr. Allem (syn. Amburana claudii Schw. & Taub.) is the only known species belonging to the genus Torresea (Amburana). This medium to large tree grows in the regions of South America between Brazil and Northern Argentina. It yields seeds known as amburana (or mamona) seeds,[1,2] which have a pronounced odour of " cumarin " (tonca bean). All parts of the tree contain coumarin,[3] more specially the seeds (0.1 to 4.0 %) and the bark. The seeds which contain an alkaloid torreseine and a bright yellow oil (22 to 28 %)[3,4] are used for flavouring tobacco and as a substitute for tonca beans. The wood is scented and in limited supply; it is highly esteemed for furniture in Argentina.

The seeds for this investigation were supplied by Dr. M. L. Meara who considered that in certain undefined respects the oil behaved unusually.

The oil was extracted with petroleum ether(b.p.40-60°c and the mixed acids have been examined by R.P.C. as such and also after hydrogenation and ozonolysis.

The seed oil has been referred to by Liberalli and Lima,[3] and Berger[4] but only the characteristics are recorded

Amburana seed oil.

Summary of results.

Seed oil.

The oval-shaped seeds (about 1.4 cm. long and 1.0 cm. wide) contain kernels which form 74 % of the total weight. The sample of extracted oil used in this investigation is compared with the previous reports on this oil.

	Liberalli & Lima.[3]	Berger[4]	Present work.
Oil.			
Content in kernels (%).	22.4	28.1	28.1
Saponification equivalent	357.8	282.2	-
Iodine value	62.3	116.6	66.5
Unsaponifiable (%)	-	-	0.8
Mixed acids excluding nonsaponifiable material.			
Saponification equivalent	-	-	288.6*
Iodine value	-	-	69.8*

* Values of 281.8 and 71.0 are calculated from the results obtained in this investigation.

Component acids from R.P.C. data given on pp. 149-152

Acids.	Mol %.	Wt. %.
Capric	1.1	0.7
Lauric	0.4	0.3
Myristic	0.8	0.6
Palmitic	12.5	11.4
Stearic	3.4	3.4
Arachidic	2.7	3.0
Behenic	3.7	4.5
Lignoceric	2.4	3.1
Hexadecenoic	4.5	4.1
Oleic	60.1	60.3
Linoleic	5.8	5.8
Linolenic	trace	trace
Eicos-9-enoic	2.6	2.8

Discussion.

1. The C_{18} monoethenoid was identified as oleic acid by converting to erythro 9:10 dihydroxy stearic acid (m.p. and mixed m.p. 129-130°C). On bromination of the mixed acids tetrabromo stearic acid (m.p. and mixed m.p. 113-114°C) and a very small amount (trace) of hexabromo stearic acid (m.p. and mixed m.p. 181-182°C) were isolated and identified as linoleic and linolenic acids respectively.

2. The C_{20} monoethenoid is probably eicos-9-enoic acid, since G.L.C. of the esters isolated from the petroleum ether extract of the ozonised esters showed the presence of a C_{11} monobasic acid.

3. The presence of the acids determined by R.P.C. in the mixed acids of Amburana seed oil has been confirmed by G.L.C. of the mixed esters before and after hydrogenation.

4. The results obtained in this investigation (presence of C_{20} and higher acids) are in general agreement with those for other seed oils belonging to the Leguminosae family.[5]

Detailed chromatographic results.

Summary of R.P.C. results. (%. mol)

Acid group	10:0	12:0	14:0	16:0	18:0	20:0	22:0	24:0
Eluting solvent% (acetone)	45	53	62	67	73	78	83	83
Hydrogenated acids	0.7	0.5	0.7	17.0	68.9	4.9	4.1	3.1
Mixed acids	1.4	0.2	11.7	72.6	7.5	2.5	4.1	?
Ozonised acids	-	0.5	0.9	12.5	3.4	2.7	3.3	1.7

These figures lead to the results on page 147. On bromination of the mixed acids, a very small amount of hexabromo stearic acid (m.p. and mixed m.p. 181-182° C) was isolated, indicating the presence of linolenic acid. The C_{18} triethenoid acid, if present, is expected to elute with the C_{12} group of acids.[6] In the present investigation, it is obseved that this acid is eluted faster than the C_{12} acid group.

Two peaks are again obtained for the C_{14} group of acids with 62 %. acetone in the mixed acids chromatogram (cf. pp. 115-117).

The ozonised acids were extracted with petroleum ether and the methyl esters of the acids isolated from this extracts were analysed by G.L.C. on a 5 %. Apiezon L. column at 150°C. using methyl laurate as standard. The G.L.C. clearly indicated the presence of a C11 monobasic

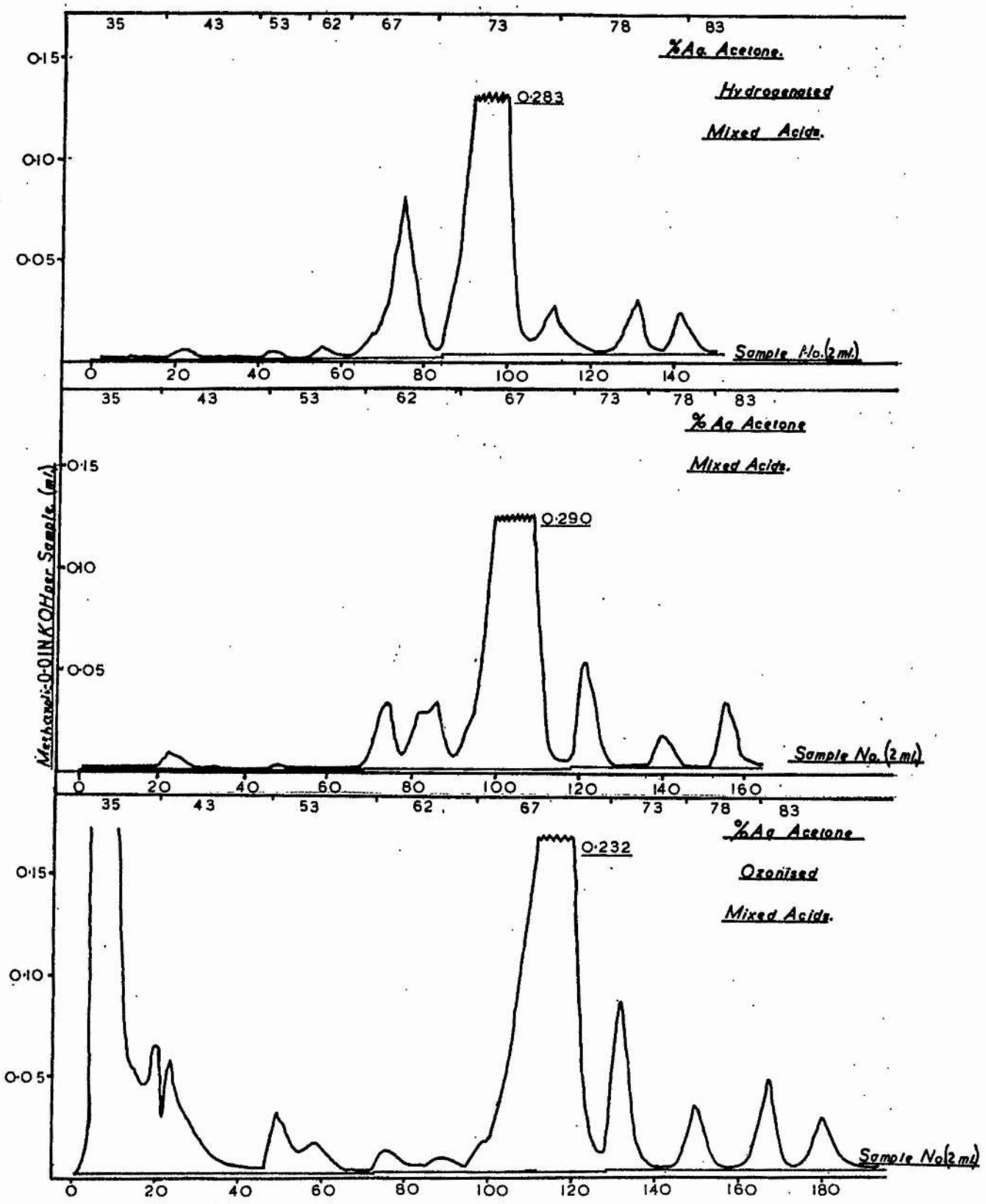


FIG.7 TORRESEA CEARENSIS (AMBURANA) ACIDS.

acid. There is good agreement between the observed (68.9%) and calculated values (69.3%) for the C_{18} group of acids.

Amburana seed oil

Detailed R.P.C. results.

Hydrogenated acids (20.8mg.), S.E.288.6, Recovery 85%.,
Alkali 1.8618 x 10^{-2} N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq x 10^{-5}	Mol%
10:0	19-26	0.031	0.008	0.023	0.043	0.7
12:0	41-47	0.024	0.007	0.017	0.032	0.5
14:0	52-59	0.037	0.015	0.022	0.041	0.7
16:0	62-82	0.588	0.042	0.546	1.016	17.0
18:0	83-105	2.298	0.090	2.208	4.111	68.9
20:0	106-121	0.322	0.064	0.158	0.294	4.9
22:0	125-136	0.179	0.048	0.131	0.244	4.1
	137-148	0.148	0.048	0.100	0.186	3.1

Mixed acids (20.3mg.), S.E.288.6, Recovery 95.5%,
Alkali 1.8618 x 10^{-2} N.

Acid group	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq. x 10^{-5}	Mol%
10:0	19-28	0.061	0.010	0.051	0.0949	1.4
12:0	45-50	0.019	0.012	0.007	0.0130	0.2
14:0	68-78	0.195	0.022	0.173	0.3221	4.8
	79-90	0.273	0.024	0.249	0.4636	6.9
16:0	91-116	2.675	0.052	2.623	4.8835	72.6
18:0	117-129	0.311	0.038	0.273	0.5083	7.5
20:0	136-146	0.122	0.033	0.089	0.1657	2.5
22:0	151-162	0.183	0.036	0.147	0.2737	4.1

Ozonised acids (36.2mg.), S.E. 288.6, Recovery 25.1%.,
Alkali 1.8618×10^{-2} N.

Acid	Fr. No.	Tot. Alk.	Blank	Alk. Corr.	Eq. $\times 10^{-5}$	Mol%.
12:0	57-61	0.045	0.010	0.035	0.065	0.5
14:0	62-71	0.092	0.030	0.062	0.115	0.9
16:0	74-99	0.972	0.082	0.890	1.657	12.5
18:0	100:112	0.298	0.052	0.246	0.458	3.4
20:0	116-130	0.251	0.060	0.191	0.356	2.7
22:0	131-142	0.286	0.048	0.238	0.443	3.3
	143-155	0.171	0.052	0.119	0.221	1.7

Examination by gas-liquid chromatography.

(i). Mixed esters before and after hydrogenation.

The mixed esters showed peaks corresponding to C'_{16}, C_{16}, unsaturated C_{18}, C_{18}, unsaturated C_{20}, C_{20}, C_{22} and C_{24} acids when put through a 2½ %. Apiezon L. column at 200° C., argon flow-rate 35.3 cm./ min., using methyl stearate as standard. After hydrogenating, the esters showed only C_{16}, C_{18}, C_{20}, C_{22} and C_{24} acids. The minor peaks in the mixed ~~acids~~ esters chromatogram indicated the presence of traces of C_{17}, C_{19}, C_{21} and C_{23} acids.

Using a 5 %. Apiezon L. column at 150° C. (argon flow-rate 33.3 cm./ min. using methyl laurate as standard), it was possible to show also the presence of C_{10}, C_{12} and C_{14} acids.

(ii). Monobasic esters found during ozonolysis.

A portion of the ozonised acids were extracted with petrol ether and the acids isolated from the petrol extract were methylated and put through the G.L.C. (5 %. Apiezon L. column at 150° C. argon flow-rate 33.3 cm./ min, using methyl laurate as standard). This chromatogram indicated t the presence of undecanoate which could arise from hexadec-5-enoate, octadec-7-enoate, or eicos-9-enoate. It seems likely, therefore, that the C_{20} unsaturated acid is the familiar Δ^{11} isomer.

References

1. Eckey, E.W., " Vegetable Fats and Oils ", p.479, Reinhold Publishing Corporation, 1954.

2. Record, S.J., and Mell, C.D., " Timbers of Tropical America", p. 368, Yale University Press, 1924.

3. Liberalli, C.H., and Lima, J., Rev. flora med. (Rio de Janeiro) 1937, 3, 341; C.A., 1937, 31, 4057; Rev. chim. ind. (Rio de Janeiro), 1939, 8, No. 86, 27-33 (275-81); C.A., 1939, 33, 8921.

4. Berger, F., Scientia Pharm., 1938, 11, 122; C.A., 1929, 35, 1528

5. Hilditch, T.P., " The Chemical Constitution of Natural Fats ", p. 228, Chapman and Hall, 1956.

6. Crombie, W.M.L., R. Comber, and Boatman, S.G., Biochem. J., 1955, 57, 309.